THE MORPHOLOGY AND TAXONOMY OF SOME FUNGI SELECTED FROM A SURVEY OF A NATURAL KAROO PASTURE

BY

CECILIA ROUX

DISSERTATION
SUBMITTED IN FULFILMENT
OF THE REQUIREMENTS

FOR

THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BOTANY

IN THE

FACULTY OF SCIENCE

AT THE

RAND AFRIKAANS UNIVERSITY
JOHANNESBURG, SOUTH AFRICA

SUPERVISOR: PROFESSOR K.T.VAN WARMELO

NOVEMBER 1985

I KNOW HOW SEDUCTIVE THE FUNGI ARE, ...

BRYCE KENDRICK Sept. 1985.

CONTENTS

ABSTRACT

The Morphology and Taxonomy of Some Fungi Selected from a Survey of a Natural Karoo Pasture
by
Cecilia Roux

Supervisor: Professor K.T. van Warmelo
Department: Botany
Degree: Ph.D.

A survey of the fungal flora of the Karoo over a period of five years has shown that this inhospitable environment contains a wealth of fungal species. Many of these fungi constitute new records for South Africa and also several previously undescribed species, the most important being the teleomorph of Pithomyces chartarum, described as Leptosphaerulina chartarum C. Roux. This survey has shown conclusively that P. chartarum, which is associated with photosensitization of sheep, can always be recovered from the veld when the appropriate isolation techniques are employed. The finding of an ascomycetous state for this fungus could explain why it has been so successful in such an arid region, providing a flush of conidia to coincide with the flush of new growth of Tribulus terrestris L., the pioneer plant associated with ' geeldikkop '.

The study of the conidiogenesis of three Coelomycetes viz. Urohendersonia platensis Speg., Tiarosporella graminis (Pirozynski & Shoemaker) Nag Raj var karoo Sutton & Marasas and Bartalinia robillardoides Tassi showed that the conidiogenous cells of these three species had only one cell wall layer. Based on the simplified cell wall structure, a new mode of conidiogenesis, thysanoblastic, is described. In this type of development a maximum of three conidia are produced by each conidiogenous cell. Development of the conidiomata and the conidiogenous apparatus was followed using light and transmission electron microscopy which showed that the conidiogenous apparatus was continually being replaced while the conidiogenous locule became enlarged.

SAMEVATTING

Die Morfologie en Taksonomie van Sommige Fungi Uitgesoek van 'n Opname van 'n Natuurlike Karoo Weiding. In Engels.

deur
Cecilia Roux

Promotor: Prof. K.T. van Warmelo
Departement: Plantkunde
Graad waarvoor proefskrif ingedien is: Ph.D.

'n Opname van die fungusflora van die Karoo oor 'n periode van vyf jaar het aangetoon dat hierdie onherbergsame omgewing 'n groot rykdom van fungusspesies bevat. Verskeie nuwe aanwinste vir Suid-Afrika asook onbeskrewe spesies is gevind, waarvan die belangrikste die teleomorf van Pithomyces chartarum, as Leptosphaerulina chartarum C. Roux beskryf is. Hierdie opname het oortuigend bewys dat met die gepasde tegnieke, P. chartarum wat met fotosensitiwiteit van skape geassosieer word, altyd uit die veld geïsoleer kan word. Die fonds van ' n askomiseet-stadium vir hierdie fungus kan verklaar waarom dit so suksesvol is in 'n besonder droë omgewing, deurdat dit 'n vloed van konidiums wat gelyktydig met die nuwe groei van Tribulus terrestris L., 'n pionierplant geassosieer met geeldikkop, kan vorm.

Die studie van die konidiogenese in die drie Coelomycetes, naamlik Urohendersonia platensis Speg., Tiarosporella graminis (Pirozynski & Shoemaker)Nag Raj var karoo Sutton & Marasas en Bartalinia robillardoides Tassi het aangetoon dat die konidiogene selle se wandlaag uit 'n enkellaag bestaan het. As gevolg van die vereenvoudigde selwandstruktuur is 'n nuwe proses van konidiogenese, thysanoblasties, beskryf. Volgens hierdie proses ontwikkel 'n maksimum van drie konidiums per konidiogene sel. Die ontwikkeling van die konidiomata en konidiogene apparaat is nagegaan en het aangetoon dat die konidiogene apparaat deurentyd vervang word terwyl die konidiogene lokule vergroot.

ACKNOWLEDGEMENTS

I acknowledge the following:

permission granted by Dr I.H. Wiese, Director, Plant Protection Research Institute, to use results obtained from several projects for degree purposes;

co-operation given by Dr P.W. Roux, Director, Karoo Region for the reseach on the fungal flora of the Karoo; discussions and assistance from Mr D. Blom and the late Mr J.H.P. Acocks; Mr A Barnhoorn and his colleagues, who meticulously took the samples and sent them off to Pretoria;

Dr L.A.P. Anderson and Mr J.A. Minné of the Chemical Toxicology Section, Veterinary Research Institute, Onderstepoort for assistance with the chemical assays of various Pithomyces isolates;

Mr J. Pollock of the Agricultural Meteorological Unit of the Soil and Irrigation Research Institute, stationed at Grootfontein Agricultural College, who kept the weather stations functioning and calculated the data monitored;

Dr Marius van Tonder, Regional Laboratory, Veterinary Services, Grootfontein and his staff, Dr Gareth Bath (now at Allerton, Natal) and Mr Frikkie Smit, who supervised the veterinary care of the sheep in the camps;

invaluable professional assistance and guidance provided by Professor J. Coetzee ably assisted by Mr C.F. van der Merwe, Electron Microscope Unit, Faculty of Science, University of Pretoria, who's expert knowledge of TEM was conclusive to the successful completion of the study of the conidiogenesis of the three Coelomycetes;

my colleagues in the Mycology Research Unit, especially technical assistance given by Mr P.J.J. van der Westhuizen, who plated out the Karoo samples and who's consistently high standard of work contributed largely to the success of the survey; Miss E. van der Westhuysen, who recognised the 'knobs' in the pure culture of P. chartarum, which turned out to be the teleomorph of this fungus and Dr A.J.L. Phillips, for assistance with the photography;

thanks to Pretoria architect Dennis Maas, who advised me on the 3-D drawings;

sincere thanks to Chris van der Merwe, who expertly printed the final photographs of the plates;

thanks to Dr B.C.Sutton, CMI, who encouraged me to study the Coelomycetes and identified cultures; Professor W.J.Jooste, Potchefstroom University, who's acute perception inspired me to look more

closely at the Coelomycetes and discussions I could have with Professor Bryce Kendrick, University of Waterloo, Canada, during his visit to Pretoria in July, 1985 and subsequent correspondence;

thanks to Professor K.T. van Warmelo and his wife Meg, who always returned my calls, to Professor for the excellent supervision, guidance and his unique brand of humour

and

thanks to my family, who provided sufficient incentive to make this the third in twelve months, after Paul and Elza's.

SECTION I

SURVEY

OF THE

KAROO PASTURE

CONTENTS

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-desert environment. Hyphomycetes and Coelomycetes represented 44,3 % and 36,9 % respectively of the taxa identified. A total of 123 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. 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.

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The Karoo is geologically part of the Great Karoo Basin which was shaped by ancient glacial and volcanic activity (Du Toit, 1954; Anderson & McLachlan, 1976; McLachlan & Anderson, 1977; Cluver, 1978). Subsequent erosion and change from swampy to arid conditions resulted in the flat and broken country of the central and eastern Cape Province now colloquially called the ' Karoo '. The descriptive Hottentot word means ' dry place ' (Nienaber, 1963).

Climatically the Karoo can be defined according to the Holridge system (Price, 1975) as semi-arid to arid and warm to cool-temperate. Characteristics are erratic and patchy rainfall and occasional unseasonal cold weather when snow can fall in the high lying areas even during mid-summer.

TABLE 1

Long-term meteorological data for Middelburg in the Karoo (data provided by the Soils and Irrigation Research Institute, Middelburg.)

Annual rainfall (mm). Mean ± standard error:	361,0 ±	15,0
Relative humidity range (%) Mean daily minimum and maximum	23,0 -	80,0
Absolute temperature range (°C)	- 12,6 -	41,4
Temperature range (°C) Mean daily minimum and maximum	- 0,5 -	30,3
Temperature range (°C) 5 cm below soil surface	- 1,4 -	45,1
Frost-free period. Range (days)	62,0 -	191,0

Free roaming herds of game have been replaced by sheep and the original vegetation largely replaced by karroid veld (Acocks, 1975 , 1979). There is general agreement that the process of deterioration is continuing with desertification advancing towards the northeast.

The flora of the Karoo is rich in species (Acocks, 1975) but the habitat is ' unpredictable ' with patches of ' temporary ' pioneer vegetation (Southwood, 1977). It is on these disturbed soils that pioneer plants such as Tribulus terrestris L. become established when the first early summer rains fall. Theiler (1918) showed that ingestion of T. terrestris, especially wilted material, was directly implicated in the etiology of the disease of sheep called ' geeldikkop ' . He reported the presence of a Colletotrichum sp. on such material and linked it to the disease as a possible cause. Subsequent feeding trials with the first South African isolate Pithomyces chartarum (Berk. & Curt.) M.B. Ellis from an outbreak of ' geeldikkop ' established the toxicity of the fungus (Kellerman et al., 1980).

As a result of this work and results obtained in New Zealand where P. chartarum from ingested grasses is responsible for the development of facial eczema (Thornton & Percival, 1959 ; Thornton & Ross, 1959) a survey was initiated to determine the incidence of P. chartarum in the veld when ' geeldikkop ' was likely to occur. During the study of the fungal flora on the samples of the natural Karoo pasture it became apparent that much information could be obtained if all fungi could be identified as far as possible. The results of this survey from 1978 up to 1982 are presented here.

LITERATURE REVIEW

Reported surveys of fungal floras include studies of the mycoflora of plants, soil and the microfungal succession involved in the decay of certain plants. As studies on fungal succession were not done in this investigation, only the literature referring to fungal populations was reviewed.

Various techniques have been used depending on the aim of the study. Leaves were surface sterilized or washed (Hering, 1965; Hogg & Hudson, 1966; Ruscoe, 1971; Lindsey & Pugh, 1976) to remove incidental colonizers on the leaves. As P. chartarum does not colonize living leaves and usually occurs as superficial conidia on exposed plant surfaces, surface sterilization is not an appropriate technique when looking for this organism.

The mycoflora of the leaf litter of Carex paniculata was studied by Pugh (1958). Fungi sporulating on leaf samples were recorded by direct observation while other samples were incubated at room temperature in moist chambers and species developing on such leaves were recorded. An additional set of leaf samples was surface sterilized and plated out on agar medium. Water content of the leaf samples was determined. Metasphaeria cumona and Stysanus stemonites were the most common fungi but superficial conidiophores of species of the Dematiaceae and Stilbaceae occurred more frequently than did species of the Moniliaceae (Pugh, 1958).

Dickinson (1967) studied the fungal colonization of Pisum leaves and found that they were not extensively colonized by filamentous fungi. At the onset of senescence, however, a dramatic increase was observed in the activities of several saprophytes and on newly dead leaves colonization proceeded even more quickly. The sampling techniques employed were: incubation of leaves in moist chambers and leaf surface impressions taken by painting the leaf with nail varnish, allowing the film to dry, peeling it off and mounting it in lacto-phenol with 0,1% acid fuchsin. Disks cut from the leaves were also washed and then plated out while in another set the washing water was diluted and aliquots plated out on potato dextrose agar. Leaves were categorized as to stage of degradation by the fungi present, and the beginning of decomposition was closely linked to the initiation of sporulation in several species e.g. Cladosporium, Stemphylium and Alternaria. Evaluating the significance and value of the various methods employed, Dickinson (1967) concluded that the washing dilution method was relatively inefficient because of two reasons: firstly the unknown toxicity of Tween 80 and secondly he suspected that the data obtained by this method may be an underestimate of the number of viable propagules which were actually present. The results obtained from the moist chamber treatment were in agreement with the theoretical aim of the method, which was to indicate the presence of potential fungal colonists.

In a review of the literature on the ecology of fungi on the surface of plant debris Hudson (1968) stated that primary colonizers of leaves were Cladosporium herbarum, Epicoccum nigrum, Botrytis cinerea, Aureobasidium pullulans and Alternaria tenuis. Coelomycetes were only present as early colonizers of plant litter and the succession ended with Penicillium spp., Mucorales, Trichoderma spp. and Basidiomycetes. Every substrate supported a specific fungal population.

Pugh & Mulder (1971) studied the mycoflora associated with living and senescent leaves of Typha latifolia. Samples of leaves were collected for a year and a half. Leaf samples were washed to remove debris and propagules on the leaves. Segments (6 mm^2) of the leaf blade were cut and incubated in moist chambers and on agar plates. The percentage of the segments colonized was noted. Cladosporium herbarum and Aureobasidium pullulans were the most frequently occurring fungi on the moribund leaves, both species increasing in frequency from May to September. Additional fungi which were often found were Phoma typharum, Epicoccum nigrum and Alternaria tenuis.

McKenzie & Hudson (1976) studied the mycoflora of rust-infected and rust-free poplar and plum leaves during decay. Several techniques were employed e.g. damp chamber incubation, washing, surface sterilization and direct incubation on agar and examination of developing fungi after 2 - 7 d. Results showed that Pithomyces chartarum was present at much higher frequencies on rust-free material than on

rust-infected material and was much lower on washed leaves and stems than on directly observed leaves and stems. The general pattern of colonization of litter by fungi was the same as for other plants, the most common species being Cladosporium spp., Alternaria sp., Epicoccum purpurascens, Aureobasidium pullulans, Botrytis cinerea and Phomopsis perniciosa.

Latch & McKenzie (1977) used a washing technique to study the fungal flora of grazed rye grass associated with outbreaks of facial eczema in Wales. The greatest number of colonies was isolated during the summer and autumn and the least during winter. Phoma spp. were the most commonly isolated fungi followed by Acremonium spp., Cladosporium spp. and Tricellula aquatica. These results are similar to those obtained by Di Menna & Parle (1970) where pycnidial fungi and Cephalosporium sp. (syn. Acremonium spp.) were found to be most common followed by Cladosporium herbarum. Latch & McKenzie (1977) noted that a mixed vegetation resulted in the isolation of a greater number of species than was detected by Di Menna & Parle (1970), who used the same methods. Isolations of P. chartarum represented 1% of the fungi found during the survey done concurrently with an outbreak and were only recovered from the rye grass and white clover, while none were found on the litter (Di Menna & Parle, 1970).

Relatively few surveys of mycofloras have been published from South Africa. Eicker (1973) studied the mycoflora of Eucalyptus maculata leaf litter. The leaves were cut into disks and strips washed, incubated and the developing fungi isolated and

identified. He found 39 per cent of the species to be common to both forest litter and soil. Papendorf & Jooste (1974) described five species of fungi from wheat field debris after isolation by the dilution plate method. The fungi were three species of Acremonium, Tricellula curvata and Ulocladium consortiale. No details regarding the total mycoflora were given. Eicker (1976) studied the mycoflora of Panicum coloratum associated with an outbreak of photosensitivity of sheep for an 11 month period. The methods employed were isolation by dilution plate, damp chamber incubation and direct plating out of plant material. The fungi on the phylloplane were studied by means of a cyclone separator. The percentages of isolates in the samples were noted. The main colonizers of plant litter were Phoma spp., Cladosporium spp., Alternaria alternata and Mucor sp.. The phylloplane was mainly colonized by species of Phoma, Penicillium, Trichoderma and Fusarium.

Bezuidenhout (1977) studied the Hyphomycetes associated with Cenchrus ciliaris L., a fodder grass, over an 11 month period. Sections of the leaves were directly plated out on to filter paper in damp chambers and agar plates. Fungi developing on the sections were noted and further isolations made. A total of 65 genera was found. Cladosporium, Alternaria, Epicoccum and Nigrospora were the genera found most often, Penicillium and Trichoderma were infrequent while no Aureobasidium isolates were detected. A smaller number of genera was found on the plant litter than on the green grass and upright dead leaves. She noted that more melanized species were found on the green leaves which were

subjected to bright sunlight.

Van der Merwe et al. (1979) studied the aerospora of an Eragrostis curvula pasture in South Africa using a Burckard spore trap for a period of nine months. The most common conidia were those of Cladosporium while conidia of Pithomyces chartarum made up 0,29% of the total count.

MATERIALS AND METHODS

The survey was conducted at the Grootfontein Agricultural College Farm, Middelburg, 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 (1978/89) and later in two camps of a hectare each (1979 onwards).

1978/79 SURVEY

The three sampling points were chosen after completion of a botanical survey of an area where Tribulus terrestris occurred. 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 of one hectare each; the one with a fair stand of T. terrestris, the other without. Five sheep were put into the camp with the T. terrestris (Camp A) and the plants were sampled as follows: the Karoo bushes, which were Galenia sarcophylla, G. procumbens, Felicia muricata and Lycium cinereum and two grasses, Eragrostis lehmanniana and Cynodon incompletus.

The other plant materials sampled were unidentified litter and T. terrestris. 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. This meant that a total of 52 weekly samples were 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 calender year.

1981/82 SURVEY

This survey was a continuation of the previous survey, which continued to the end of March 1982.

METHODS

Samples were taken up to a height of 15 cm 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 would be sun-dried before packing into paper bags, every sample from each species of plant packed separately and locality, date and species noted. They were then sent off to Pretoria by post. This distance of 800 km was covered in approximately 10 days.

Upon arrival in Pretoria, the material was sorted and cut into lengths of one cm. Individual leaflets or pinnae were used as a sampling unit. Pinnae from as many different leaves as was possible were taken from each sample. Fifty units from each of the samples were plated out on Potato Carrot agar (PCA) (CMI, 1983) to which 125 mg Albamycin T (Upjohn) had been added prior to autoclaving. The plates were incubated for a period of seven days at 24 °C with intermittent uv and daylight fluorescent light from a height of 50 cm 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 of P. chartarum (Marasas et al.,1972) were done on a number of the isolates. Some of these cultures were also used to produce bulk cultures with which to dose sheep.

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 thermohygrograph to record the daily maximum and minumum temperature, an anemometer, manual and automatic rainfall meters and a grass minimum thermometer.

VETERINARY ASPECTS

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 ".

MICROSCOPY

The fungi were initially identified at magnifications of 25 x and 50 x using a Zeiss stereomicroscope. Verifications of identifications were done with a similar make of research microscope. Material was mounted in lactophenol (CMI, 1983) but from 1980 the coelomycetes were mounted in ammonium hydroxide with 3,5% erythrosin (Sutton, 1980). Photomicrographs were made with an Olympus microscope camera and Ilford Pan F film.

SAMPLING METHODS WHICH PROVED INAPPROPRIATE

SPORE TRAPPING

A Burckard volumetric spore trap was operated from 26 January 1976 to 26 February 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.

EXPOSURE OF PETRI DISHES

This technique had the dual advantage that it gave the best indication of how many airborne conidia there were and also that isolates obtained were alive and could be used for sporidesmin assays almost right away.

However, the distance between Grootfontein and Pretoria made this an impracticable method. It was noted that under windy conditions the petri dishes could be opened for 10 min whereas 20 min 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 many fewer conidia could be collected. P. chartarum was collected in every petri dish exposed.

WASHED PLANT MATERIAL

Washing was done by shaking material in tap water mixed with Teepol (Shell Chemicals) 1:100 in a wrist shaker for 10 min to dislodge conidia. The washed material was plated out directly afterwards. The first five samples collected during the 1980/81 survey were studied in this way. The results are given in Table 2 :

TABLE 2

Percentage of pieces of plant material contaminated with P. chartarum after being washed thoroughly.

Plant Material	Camp A	Camp B
Plant litter	3,7	4,0
T. terrestris	Not	Available
Cynodon incompletus	2,0	2,8
Eragrostis lehmanniana	3,0	4,4
Lycium cinereum	6,0	2,4
Galenia procumbens	3,0	1,5
Galenia sarcophylla	9,3	16,0
Felicia muricata	0,6	4,0

THE VEGETATION SAMPLED

Tribulus terrestris L.

T. terrestris , known as caltrop in the U.S.A., three-cornered jack in Australia and also as Mexican sand-burr (Watt & Breyer-Brandwijk, 1962) is notorious for causing disease in sheep and goats. 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.

'Geeldikkop' was first described by Hutcheon (1886). In 1918 Theiler proved that T. terrestris was responsible for ' geeldikkop ', but very few researchers have been successful in reproducing ' geeldikkop ' under field conditions (Van Tonder, Basson & Van Rensburg, 1972). Kellerman et al.(1980) were able to show that the combination of Pithomyces chartarum and Tribulus terrestris gave histopathological lesions similar to those found during natural outbreaks of the disease.

It is important to note, however, that the local farmers talk of ' toxic camps '. This implies that T. terrestris growing in certain areas would always be toxic, whereas elsewhere it would be harmless.

T. terrestris (Fig. 1 a) is an annual pioneer plant with a sprawling habit which forms a ground cover. Under exceptional conditions it may appear to adopt a climbing habit. It is thus the first step in the re-establishment of permanent vegetation on denuded areas. It can, however, represent an ecological climax when it occurs in flood-plains

were the nutrients have been washed out.

Morphologically the plant has composite leaves consisting of up to 11 small pinnae. These pinnae are covered adaxially with long unicellular hyaline hairs which are arranged parallel to the surface of the leaf away from the central axis of the plant. The abaxial surfaces of the leaves have no special adaptations. These hairs act as a mirror to reflect the sun. Nevertheless, temperatures in the leaves can reach 60° C (L.A.P. Anderson, pers. comm.).

Galenia sarcophylla Fenzl

This is a herbaceous sprawling ground cover (Fig. 1 b) with a semi-succulent appearance. It is extremely palatable to grazing animals. The leaves have an unusual morphology, the epidermis having developed special unicellular and multicellular hairs with which the rays of the sun can be reflected. G. sarcophylla is preferred to T. terrestris by grazing sheep and fills the same ecological niche as T. terrestris.

Galenia procumbens L.F.

This is a perennial erect plant (Fig. 1 c) with woody stems and small smooth simple leaves. It is a hardy shrub growing to a possible height of 50 cm and is highly palatable to sheep.

Felicia muricata Thunb.

A member of the Asteraceae, this multistemmed perennial plant (Fig. 1 d) has simple leaves with a sticky surface which serves as an ideal spore trap. The plant is highly palatable to grazing animals.

Lycium cinereum Thunb. sensu lato[1]

This is an erect perennial plant, preferentially grazed in the young stages but shunned when older and harder, because of its thorny nature. The woody branches can reach .a height of 1 m plus, but when grazed heavily this species is included among the smaller Karoo bushes (Fig. 1 e). It has simple, smooth leaves and produces small red berries after flowering in January.

GRASSES

Two grasses were present in the areas covered in this survey:

Cynodon incompletus Nees

C. incompletus is an annual ground cover with a sprawling habit similar to T. terrestris and G. sarcophylla (Fig. 1 f). The leaves are covered with unicellular hairs and typically grooved because of the parallel venation.

Eragrostis lehmannianaNees var lehmanniana

This is an erect tussock grass which is intensively grazed (Fig. 1 g). It is a perennial but can become annual depending upon the prevailing conditions.

RESULTS

VEGETATION

1978/79 SURVEY

The botanical survey indicated three areas which were designated as points A, B and C where T. terrestris was present in different quantities. The nature of the three communities regarding crown, basal cover and density varied significantly.

Point A was situated in a community which consisted of a reasonably high density of perennial Karoo bushes. Therefore, the crown cover was such that wind movement between the individual bushes was possible. A fair basal cover of T. terrestris was present which diminished 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 G. 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.

1979/80 SURVEY

Because changing weather patterns adversely effected the re-establish-

ment of plants at the three points, it was decided to select two camps of one hectare each where the study could be continued. Here the replacement of T. terrestris by Salsola kali L. , an unpalatable, smooth-surfaced plant and Salvia sp. had not proceeded as far as at the three previously selected points.

The vegetation in Camp A consisted of an open, slightly build-up vegetation interspersed with patches of T. terrestris and bare soil. Sheep were introduced into this camp to graze upon the T. terrestris. However, they preferred the G. sarcophylla and consequently failed to show photosensitivity as a result of the varied diet available to them.

Initially Camp B contained very little T. terrestris and because of the well established stands of Felicia muricata and Lycium cinereum in the camp no further invasion of T. terrestris or other pioneers was possible. The density of the communities in Camp B was much higher than that of camp A, so that 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 these two camps, which indicates that these plant communities did not represent a true climax for this region. The communities in the area can thus be described as being unstable, and could, depending upon the prevailing weather, tend to become more permanent.

1980/81 SURVEY

Vegetation in Camp A deteriorated further because the summer rains,

which should have stimulated germination and growth of T. terrestris, came too late so that Salsola kali once again became the most successful pioneer plant. S. kali is only grazed in the immature state as it eventually rots off at ground level and blows about.

Camp B became an even more permanent community with very few T. terrestris plants. Felicia muricata became even more significant and the crown closed up considerably. Eventually T. terrestris was found only in the north-western corner of the camp and could not be sampled continuously.

1981/82 SURVEY

Among farmers in the region it is considered a fact that there are 'toxic' and 'non-toxic' camps. Whether this is so has not been proven. However, certain factors seem to be common to all these so-called 'toxic' camps; they are situated in flood-plains with sandy soils, have very few permanent plants and are subjected to hoof action which looses soil and exposes the lower humus layers in the soil, together with their micro-organisms. A temporary movable camp of one hectare was, therefore, established in a known toxic camp where 'geeldikkop' was known to have occurred (Van Tonder et al.,1972).

The existing vegetation was removed in order to give T. terrestris an advantage in establishing itself before other plants could do so. However, the rains came too late, which resulted in the development of too few plants to warrant the continuation of the survey. Only three samples were collected and very few fungi were found on them.

THE FUNGI

The fungi identified are listed in Table 6 and new records of genera and species for South Africa are indicated. The main groupings of incidences are given in Table 3 and a summary of the sampling dates and numbers of samples collected are given in Table 4.

A summary of the percentages of occurrence of the different taxa identified is given in Table 5. Tables 7, 8 and 9 give complete information regarding the percentages of occurrence of most identified fungi on particular substrates for the surveys from 1978 to 1981.

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

Seasonal fluctuations characterised most of the more prevalent fungi as indicated in Fig. 7 through Fig36. The seasonal incidences have been summarized in Table 3 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. The weather kept to the same pattern over the entire survey and, therefore, the record for the whole year which was sampled is given (Fig. 4).

Average occurrences of the dominant fungi at the various sampling points and areas are presented for the Hyphomycetes (Fig. 5),

for the Coelomycetes and the genus Leptosphaerulina (Fig. 6), the only ascomycete which occurred continuously, for the periods 78/79, 79/80 and 80/81.

Sudden fluctuations can be attributed to personal sampling error when someone who did not do this regularly would do the sampling.

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 semi-synthetic broth (Di Menna, Campbell & Mortimer, 1970) for three weeks under uv and daylight fluorescent tubes on a 12h/d cycle from a height of 30 cm at 20°C. The extraction procedure described by Marasas et al. (1972) was used. A total of 36 isolates or 7,5%, was positive and the highest yield was 40 mg/l sporidesmin. Most isolates, however, gave 10 mg/l or less sporidesmin under these conditions.

VETERINARY RESULTS

Although sheep were kept in atleast one sampling 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 according to Crawley & Wool= ford (1965) had occurred.

DISCUSSION

When evaluating this survey, it is important to note that of the 12 coelomycete entries in Doidge (1950), virtually all were collected by MacOwan from Somerset East in the Eastern Cape. The host plant Tribulus terrestris, on which much of the attention in the present study was focussed, is not mentioned at all in her list of host plants. The fungi which Doidge (1950) had recorded for the other plants are mainly Basidiomycetes, such as Puccinia lyci Kalchbr. and P. galeniae Diet. The fungi from litter in the Karoo have never received any attention before. The only other work in this area resulted in the description of the new species Pithomyces karoo Marasas & Schumann (1972), but the complete results of what was found were never published.

This survey gives complete information regarding the fungi present in a natural Karoo pasture over several years.

The most interesting finding was that the Coelomycetes were prevalent and diverse although the numbers found were not comparable to those of the Hyphomycetes (Table 7, 8). The 45 genera of identified Coelomycetes and 55 genera of the Hyphomycetes included 24 genera of the Coelomycetes and 8 genera and 10 species of Hyphomycetes as new records for South Africa. Furthermore, two new records of Ascomycetes were noted, including one new species, Leptosphaerulina chartarum, which

is the teleomorph of Pithomyces chartarum.

The total of 55 known genera of Hyphomycetes found in this survey is not as low as it would appear to be 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. Pugh and Mulder (1971) 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 increasing dry conditions. Ascochyta spp. and Camarosporium spp. increased with time and then levelled off. The incidence of Drechslera spp., Epicoccum purpurascens and Pithomyces chartarum declined over the years studied, although these organisms still occurred consistently. The only Ascomycete which occurred consistently was the genus Leptosphaerulina which also declined eventually.

The inescapable conclusion is that the plant community studied contains a wealth of fungi, many as yet unrecorded. Noteworthy was the occurrence of albino strains of the common Alternaria alternata, Cladosporium cladosporioides and Stachybotrys chartarum.

The Hyphomycetes were not as restricted regarding substrate as the other groups encountered and occurred widely. Unusually low incidences were, however, noted for species of Trichoderma, Penicillium and Aspergillus (Table 7).

Those Coelomycetes which did have a wide substrate distribution range were the following : Ascochyta spp., Camarosporium spp., Diplodia spp. and Phoma spp. (Table 8).

The highest incidence of most genera was noted during the autumn and winter (Table 3). This could be explained by the fact that free water in the form of dew and rain was available for longer periods of time, thus enhancing the growth of fungi (Fig. 4). Grass minimum temperatures recorded were substantially lower in winter than in summer. Highest rainfall usually occurred in autumn while the wind generally subsided (Fig. 4) 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 substrates studied.

The entomophagous fungi Beauveria bassiana and Metarhizium anisopliae were frequently found although in very small numbers. The occurrence of these fungi could be related to the emergence of the Karoo caterpillar, Loxostege frustalis Zeller, which is parasitized by B. bassiana (Möhr, 1982). This fungus was more prevalent towards the third year of the survey at which time the Middelburg district had been experiencing drought conditions for the third successive year. Associated with the absence of grasses in the pastures (non-hosts of the moth), the incidence of the Karoo moth had reached epidemic numbers. As more moths emerged the soil became less compacted because of fewer plants and subsequent wind and hoof action which disturbed the soil, thus setting the conidia of B. bassiana free into the atmosphere. According to Möhr (1982) B. bassiana is the most important natural enemy of the moth and attributed to the successful control of the moth in some areas to the action of this fungus. L. frustalis feeds mainly on Pentzia spp. and other typical Karoo bushes which were not found in the areas studied in this survey. This could explain the low incidence of B. bassiana in the areas studied during this survey.

The increase in number of spedes of Hyphomycetes from 1980 onwards

can also be explained by 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 other plant material available at the various sampling points and thus included all plant species not sampled separately. It is, therefore, understandable that it should be much more rich in variety than actual plants sampled individually.

One species which deserves special mention is Aspergillus flavus. This toxigenic fungus was very common in animal feeds from all over South Africa examined for mycotoxicological fungi during the same period (C. Roux, unpubl.), whereas it was not present during the normal rainy seasons, e.g. 1978/79.

Due to the large number of samples and the primary emphasis on Pithomyces chartarum, species of common genera such as Fusarium and Drechslera were not recorded separately. The most common species in Fusarium was F. moniliforme followed by F. subglutinans. In the genus Drechslera the following species were identified: D. halodes, D. hawaiiensis, D. papendorfii, D. phlei, D. rostrata, D. cynodontis and D. carbonus. This sequence represented their frequency of occurrence, D. halodes being the most prevalent. Members of the Drechslera-Bipolaris-Helminthosporium-complex were grouped together as Drechslera.

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 facial eczema in sheep. The same conditions were assumed to be necessary for the development of ' geeldikkop ' in local sheep. No such conditions were detected and no cases of photosensitization on the sampled pastures were reported.

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 reported in 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. It can, however, be assumed that L. chartarum was more prevalent at times when incidences of P. chartarum reached peaks, e.g. late summer and early winter, as both fungi were more prevalent then (Fig.29 A & B , 34 A & B).

The survey highlights the wealth and variety of fungi found in this inhospitable environment. The large number of genera found is due to the wide range of materials sampled. A peculiarity was that virtually the same number of genera of the Coelomycetes and Hyphomycetes were found. This is the first report in which such a high proportion of Coelomycetes is reported. The presence of Coelomycetes could be an adaptation to the local conditions insofar as hyaline conidia remain protected within the conidiomata. A similar protective mechanism occurs in the Hyphomycetes as a great proportion of the species present have melanized conidia.

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.

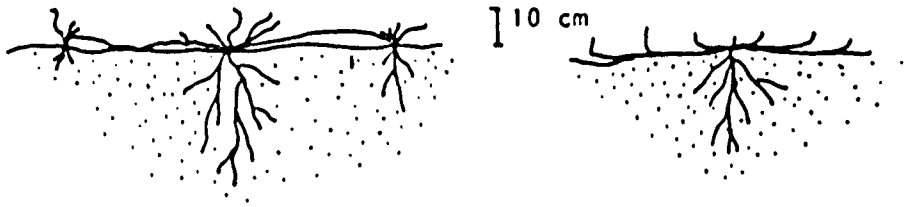
This survey demonstrated the persistent presence of Pithomyces chartarum on various substrates in the Karoo, which is a very important finding in terms of its toxicity. The fact that the teleomorph of this fungus, Leptosphaerulina chartarum, was found during this survey illustrates the importance of intensive studies of fungal populations. Had this survey been restricted to P. chartarum only, a wealth of information, particularly regarding new records and undescribed types, would have been lost.

Fig. 1 Schematic presentation of the plant types

PIONEER PLANTS

a Tribulus terretris

b Galenia sarcophylla

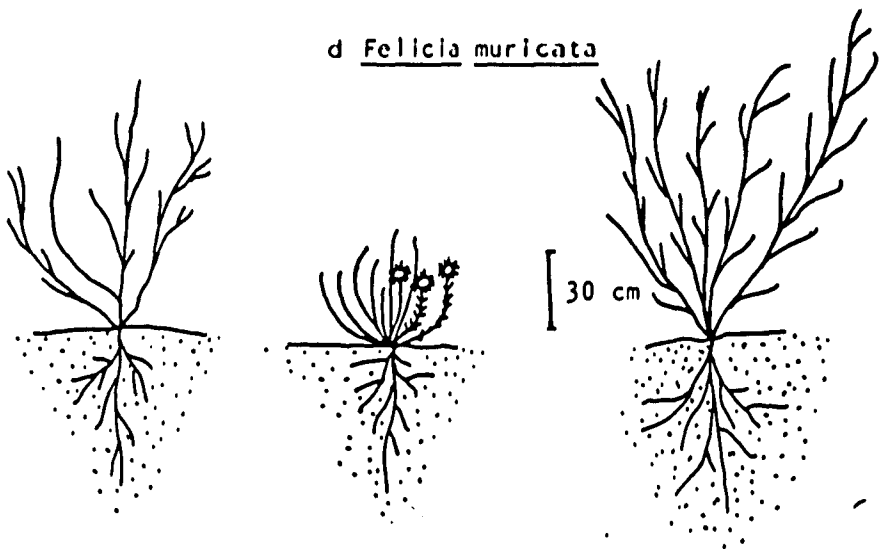


UPRIGHT 'BUSHES'

c Galenia procumbens

e Lycium cinereum

d Felicia muricata



GRASSES

g Eragrostis lehmanniana

f Cynodon incompletus

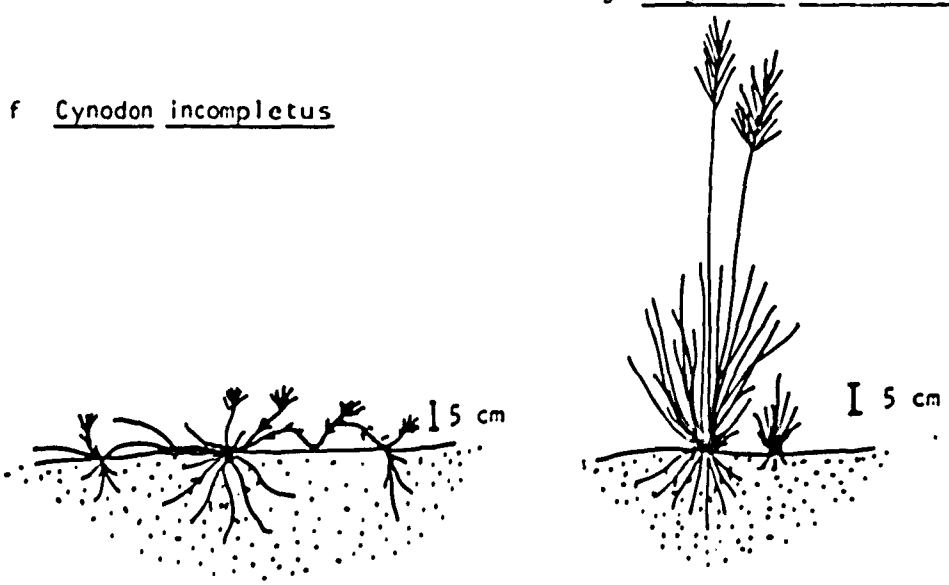


Fig 2 Hyphomycetes from the fungal survey of the Karoo

All Bars = 10 µm except Fig. 2e where Bar = 50 µm.

a Cladorhinum foecundissimum :distinct collarette on the phialide and conidia in mucilaginous ball

b Beauveria bassiana : conidiogenous cells where denticles bearing conidia are shown

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 a blade of Cynodon incompletus

f Helicomyces roseum : hyaline helicospores on conidiophore

g Volutina concentrica : stipe, setae and conidia shown in this coelomycete-like fungus

h Gyrothrix flagella : flagellum-like recurved setae in whorls

i G. flagella : conidiogenous cells at the bases of the setae

j Taeniolella sp.: characteristically curved conidia

k Curvularia tuberculata : conidiogenous cell bearing conidium with tubercles

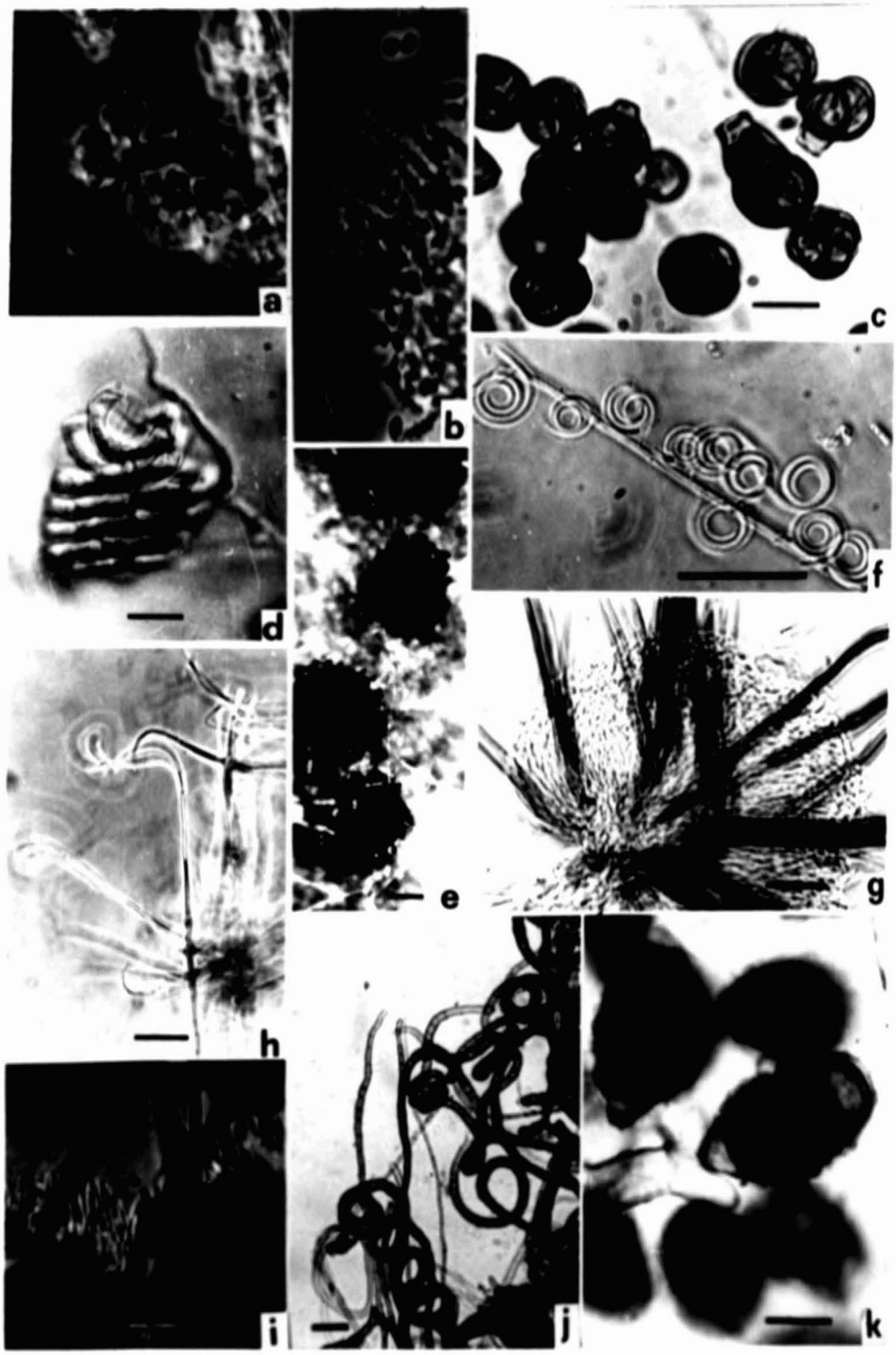

a
b
c
d
e
f
g
h
i
j
k

Fig. 3 Coelomycetes from the Fungal Survey of the Karoo

a Chaetospermum chaetosporum : conidia with hilum and appendages on apical and basal ends of the conidium (Bar = 50 µm)

b Melanophoma sp. : conidia which show the distinct epispore (Bar = 10 µm)

c Dinemasporium sp.: conidiogenous apparatus where the collarette on a phialide can be seen at the base of the protruding conidium (Bar = 10 µm)

d Dinemasporium strigosum: conidia showing the apical and basal appendages (Bar = 10 µm)

e Septoriella junci: conidium with apical mucilaginous appendage and septa clearly visible (Bar = 10 µm)

f Pyrenochaeta sp. : longitudinal section through a pycnidium showing setae surrounding the ostiole (Bar = 50 µm)

g Pseudoseptoria sp.: falcate conidia (Bar = 50 µm)

h Pseudoseptoria sp.: conidiogenous cell showing developing conidium and (inset) the characteristically long neck with multiple annellations (Bar = 10 µm)

i cf. Tetranacrium sp. : conidium with more than the usual number of divergent arms (Bar = 10 µm)

j Sarcinulella sp.: pycnidium with characteristic tendril of conidia enveloped in a mucilaginous tube (Bar = 50 µm)

k Sarcinulella sp.: detail of the conidial tendril showing constriction caused by the individual sac (arrowed) (Bar = 10 µm)

l Pestalotiopsis sp.: conidium with apical three-armed appendage and single basal appendage (Bar = 10 µm)

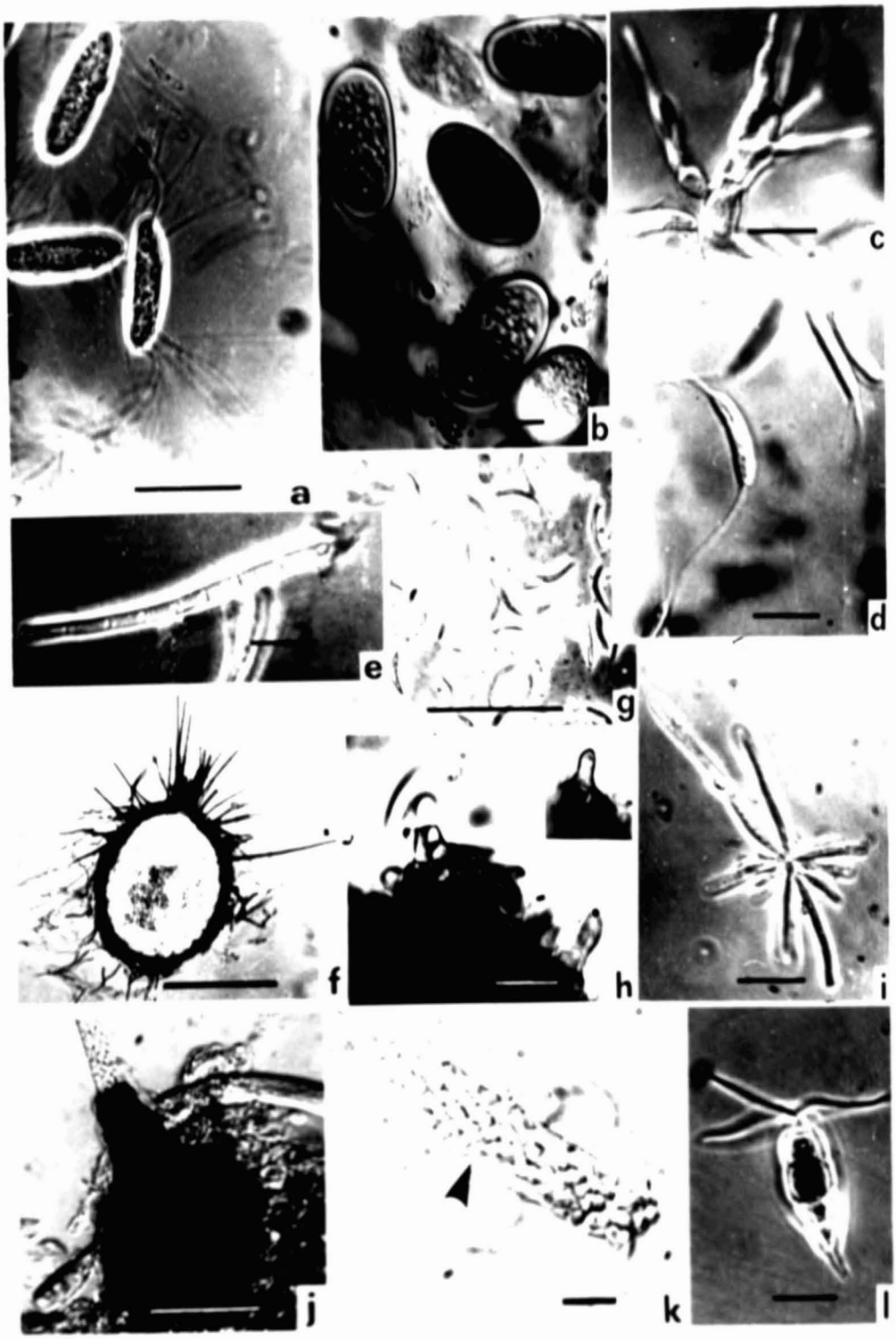

a
b
c
d
e
g
f
h
i
j
k
l

Fig. 4 Weather data for 1980/81

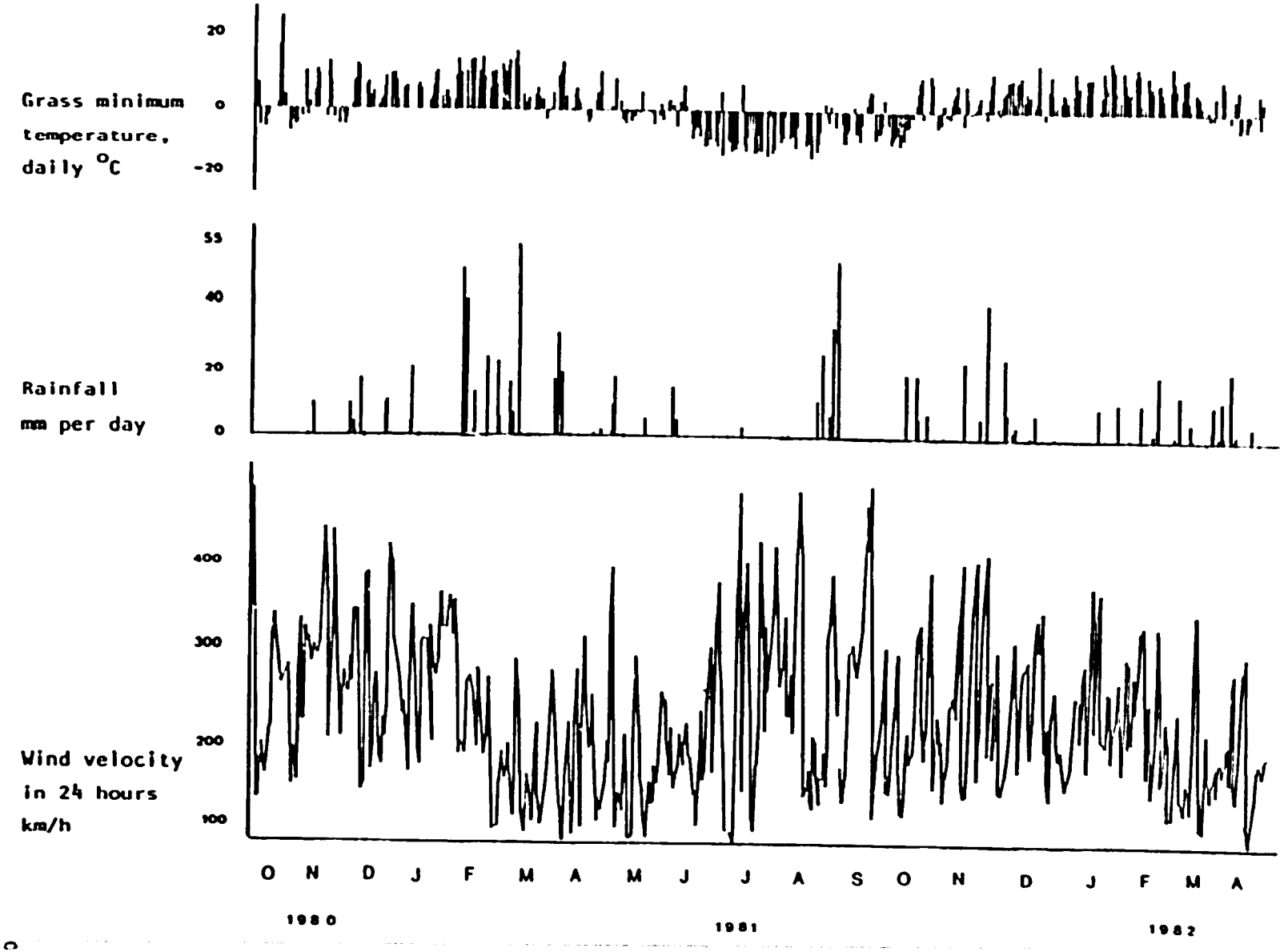


Fig. 5 Incidence of the most prevalent Hyphomycetes during the years 1978 to 1981

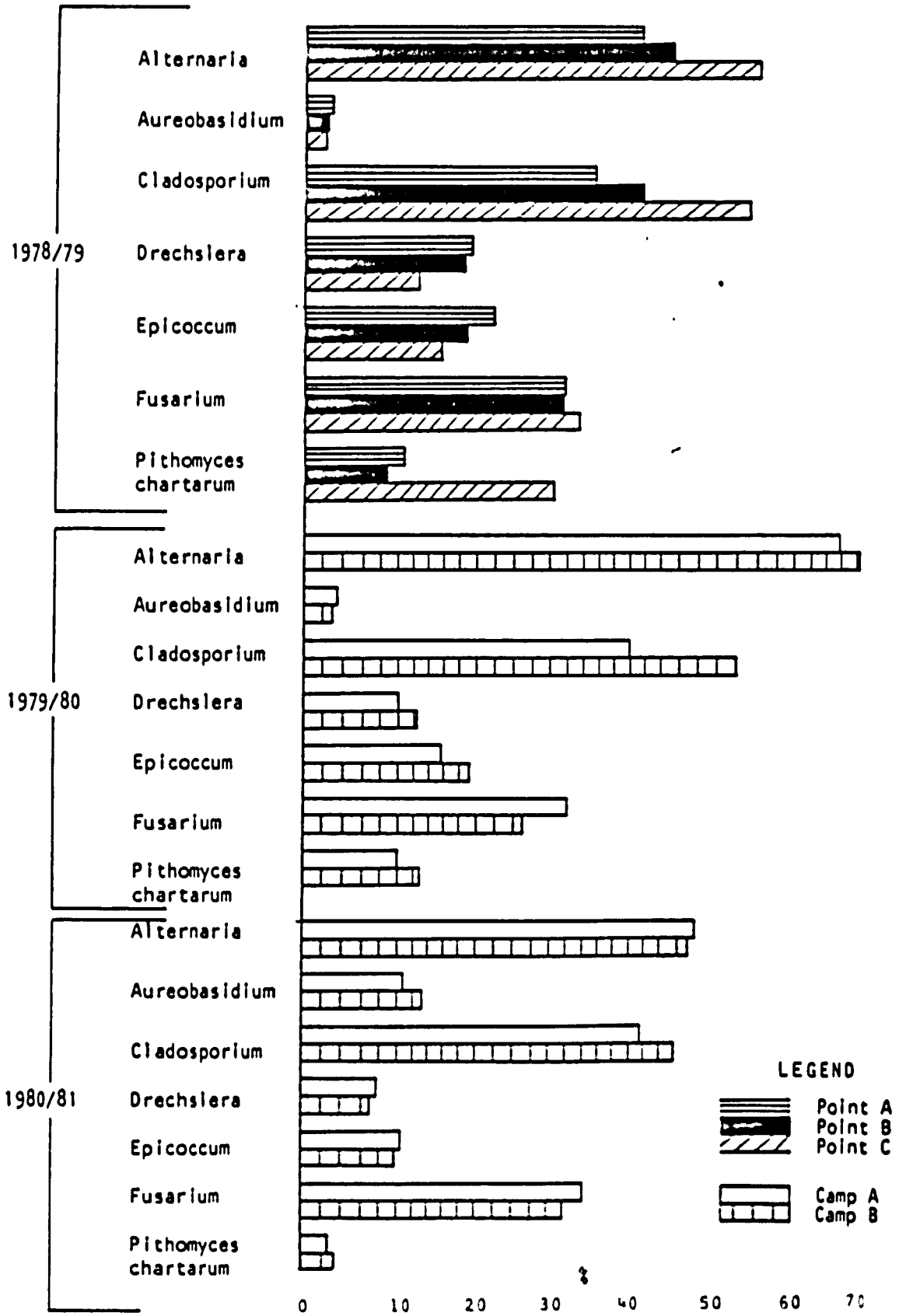
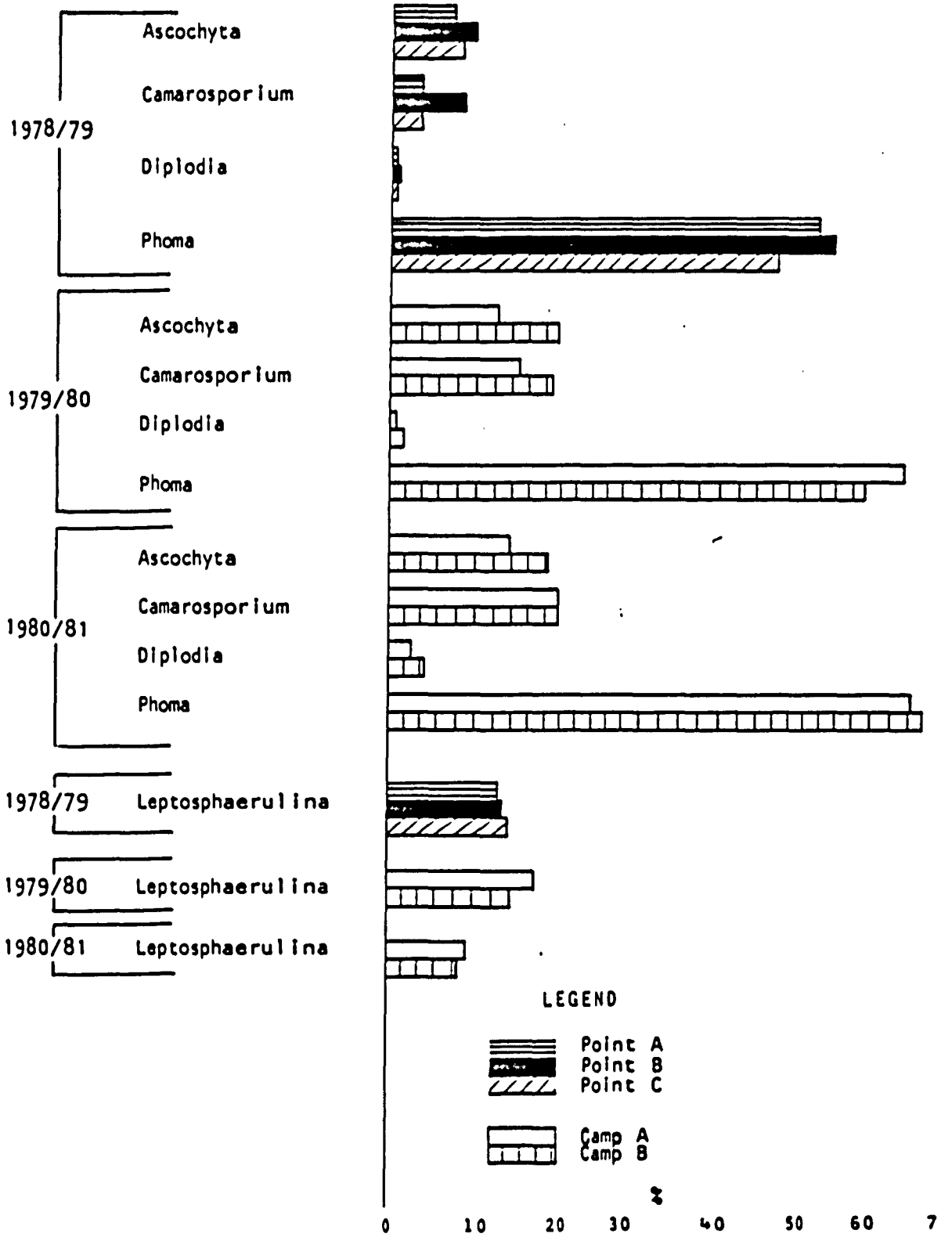


Fig. 6 Incidence of the most prevalent Coelomycetes during the years 1978 to 1981 including Leptosphaerulina spp. the only Ascomycete.



Figs. 7 - 17

Incidence of selected fungi at Points A, B & C from August 1978 to Arpil 1979 as a percentage of the samples

LEGEND

_____ litter fraction

------- Tribulus terrestris fraction

Fig. 7

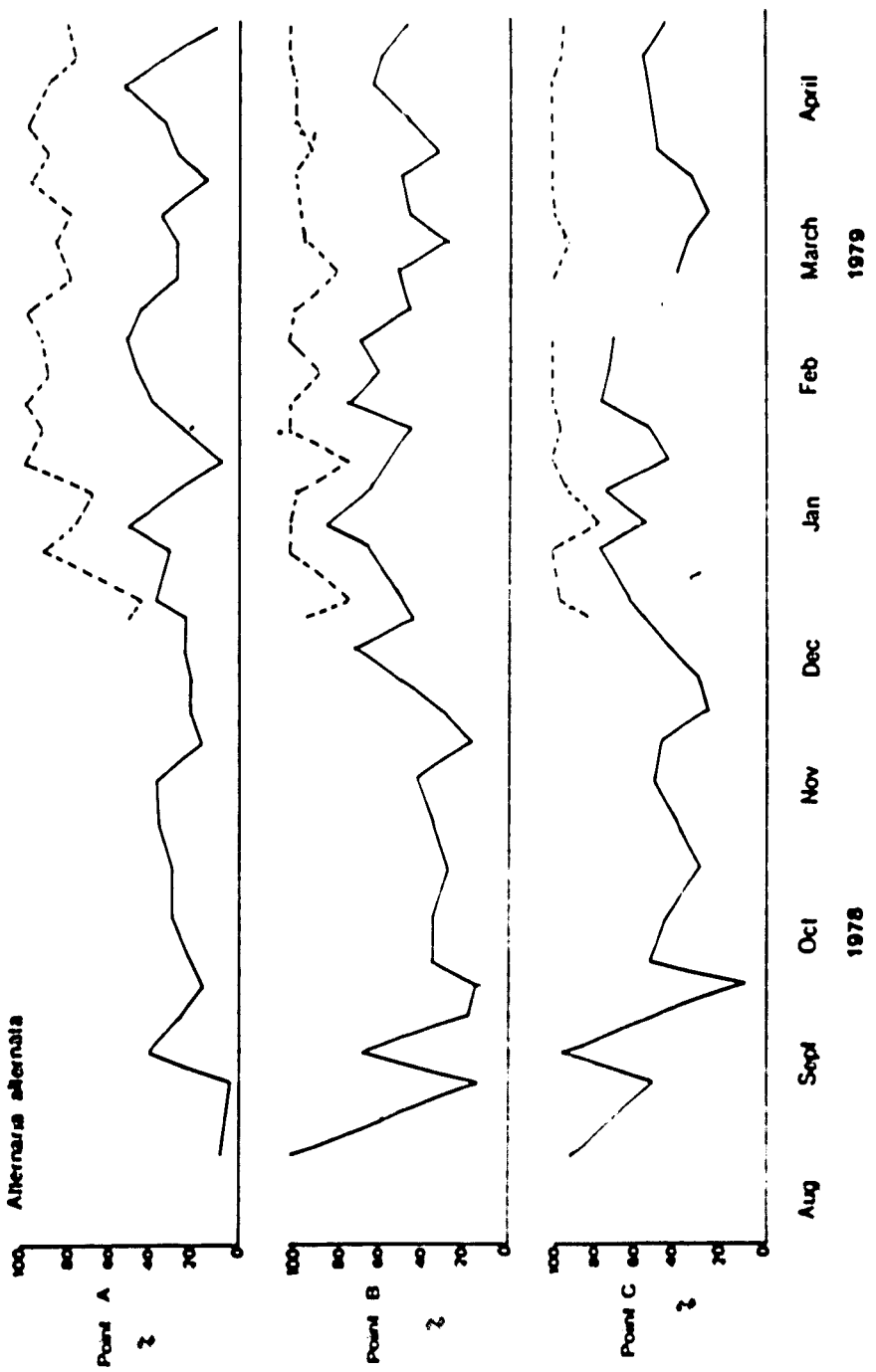

Alternaria alternata
Point A
%
100
80
60
40
20
0
Point B
%
100
80
60
40
20
0
Point C
%
100
80
60
40
20
0
Aug
Sept
Oct
Nov
Dec
Jan
Feb
March
April
1978
1979

Fig. 8

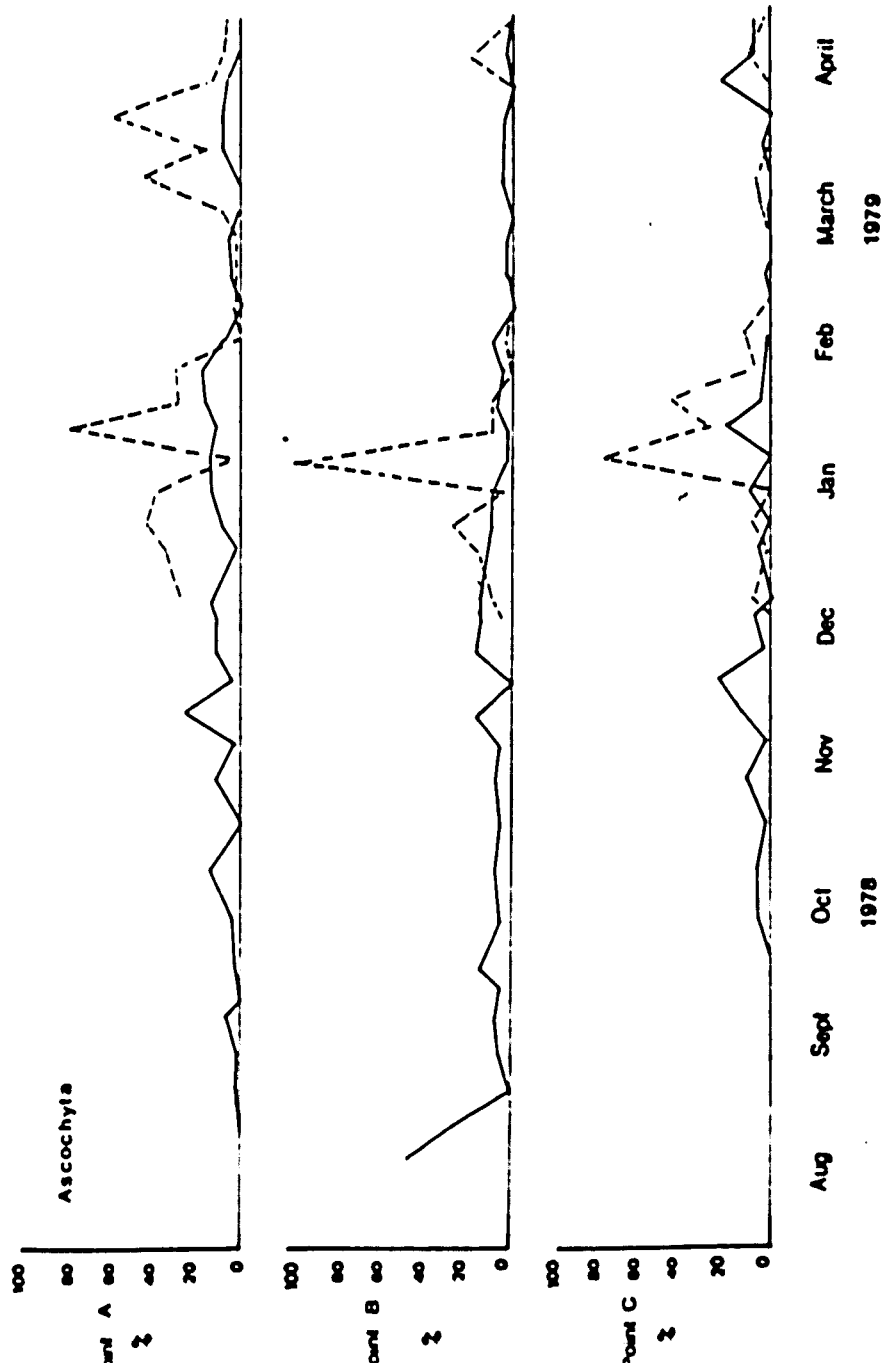

Ascochyta
Pond A
Pond B
Pond C
%
100
80
60
40
20
0
Aug
Sept
Oct
Nov
Dec
Jan
Feb
March
April
1978
1979

Fig. 9

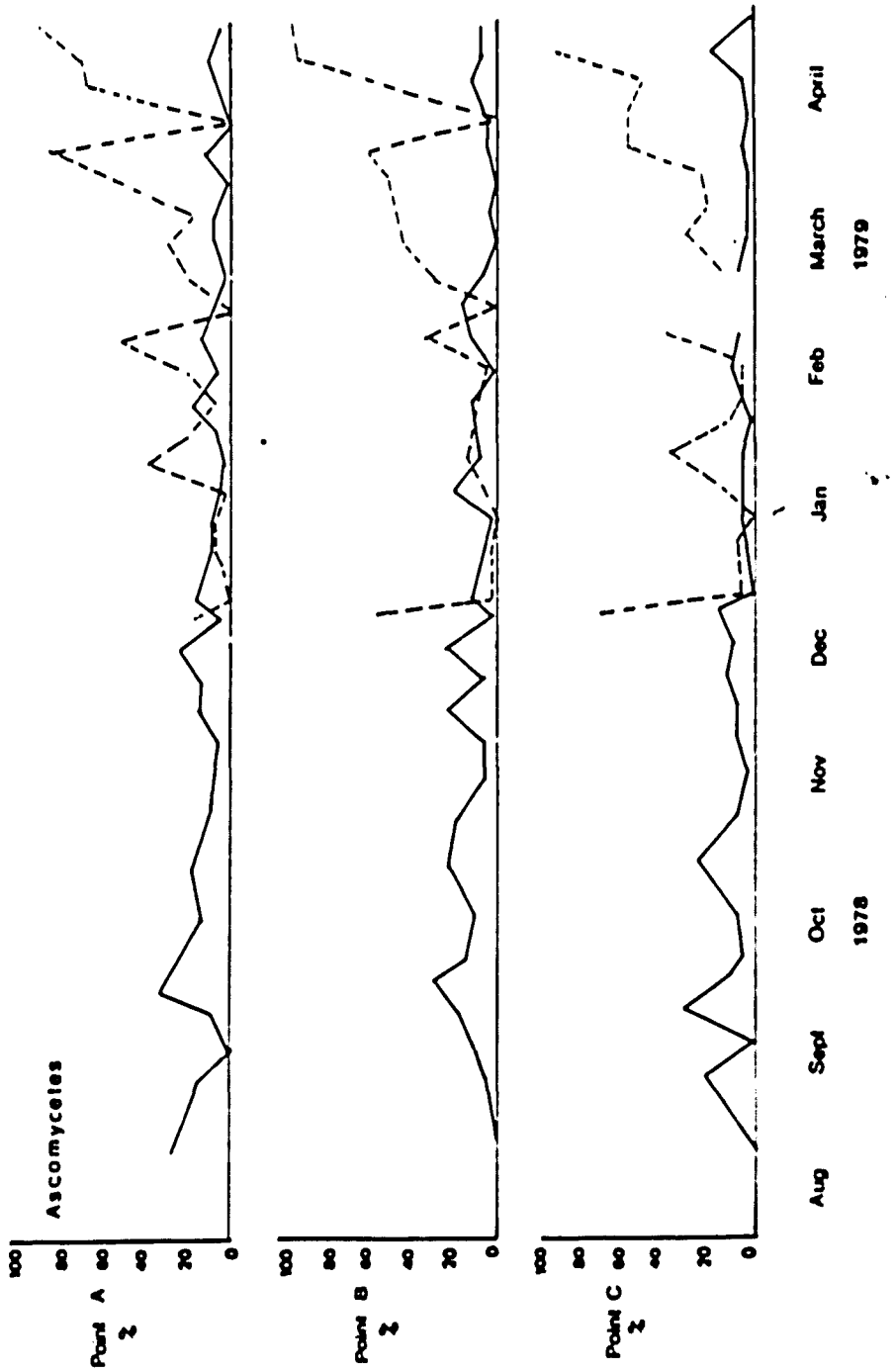

Ascomycetes
Point A
%
Point B
%
Point C
%
100
80
60
40
20
0
Aug
Sept
Oct
Nov
Dec
Jan
Feb
March
April
1978
1979

Fig. 10

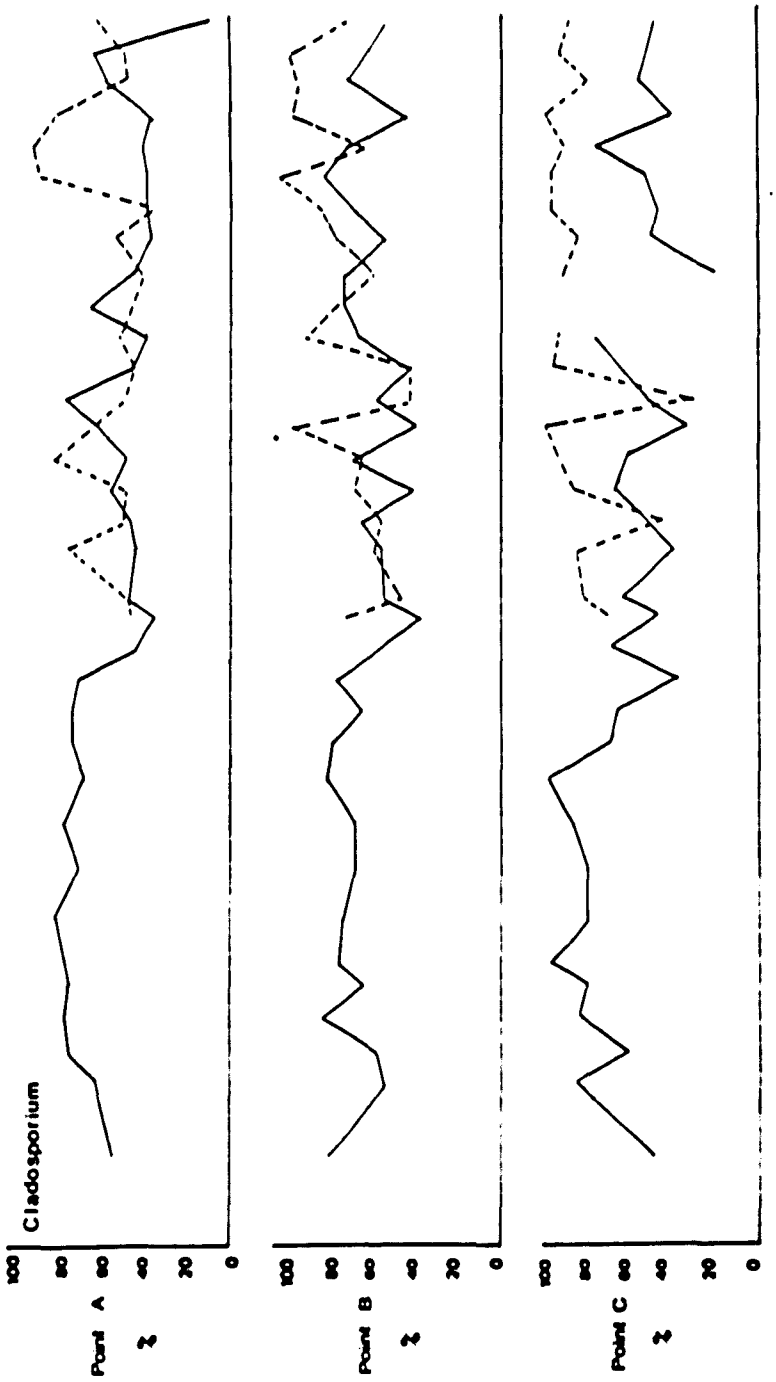

Cladosporium
Point A
Point B
Point C
%
100
80
60
40
20
0
Aug
Sept
Oct
Nov
Dec
Jan
Feb
March
April
1978
1979

Fig. 11

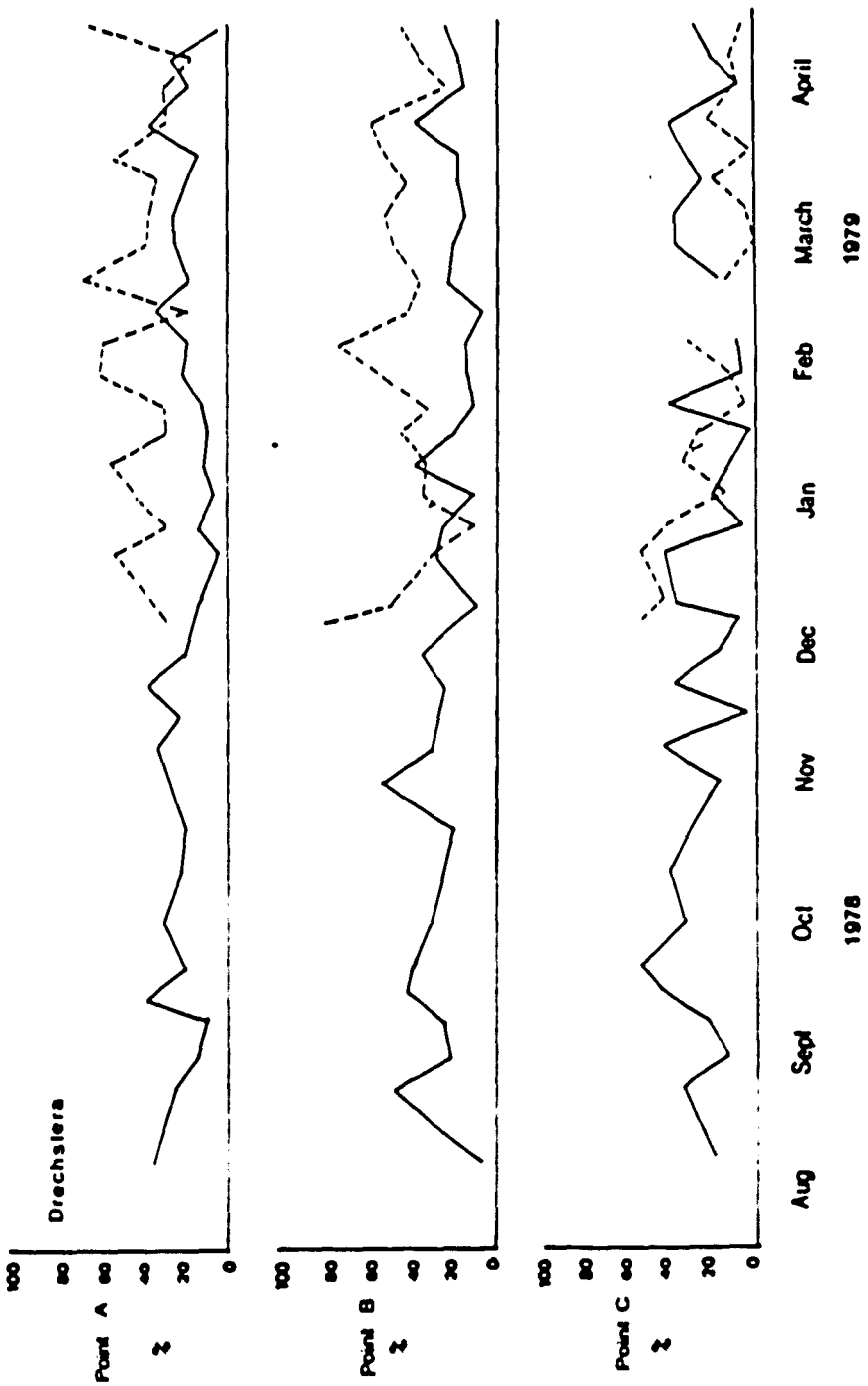

Drechslera
Point A
Point B
Point C
%
100
80
60
40
20
0
Aug
Sept
Oct
Nov
Dec
Jan
Feb
March
April
1978
1979

Fig. 12

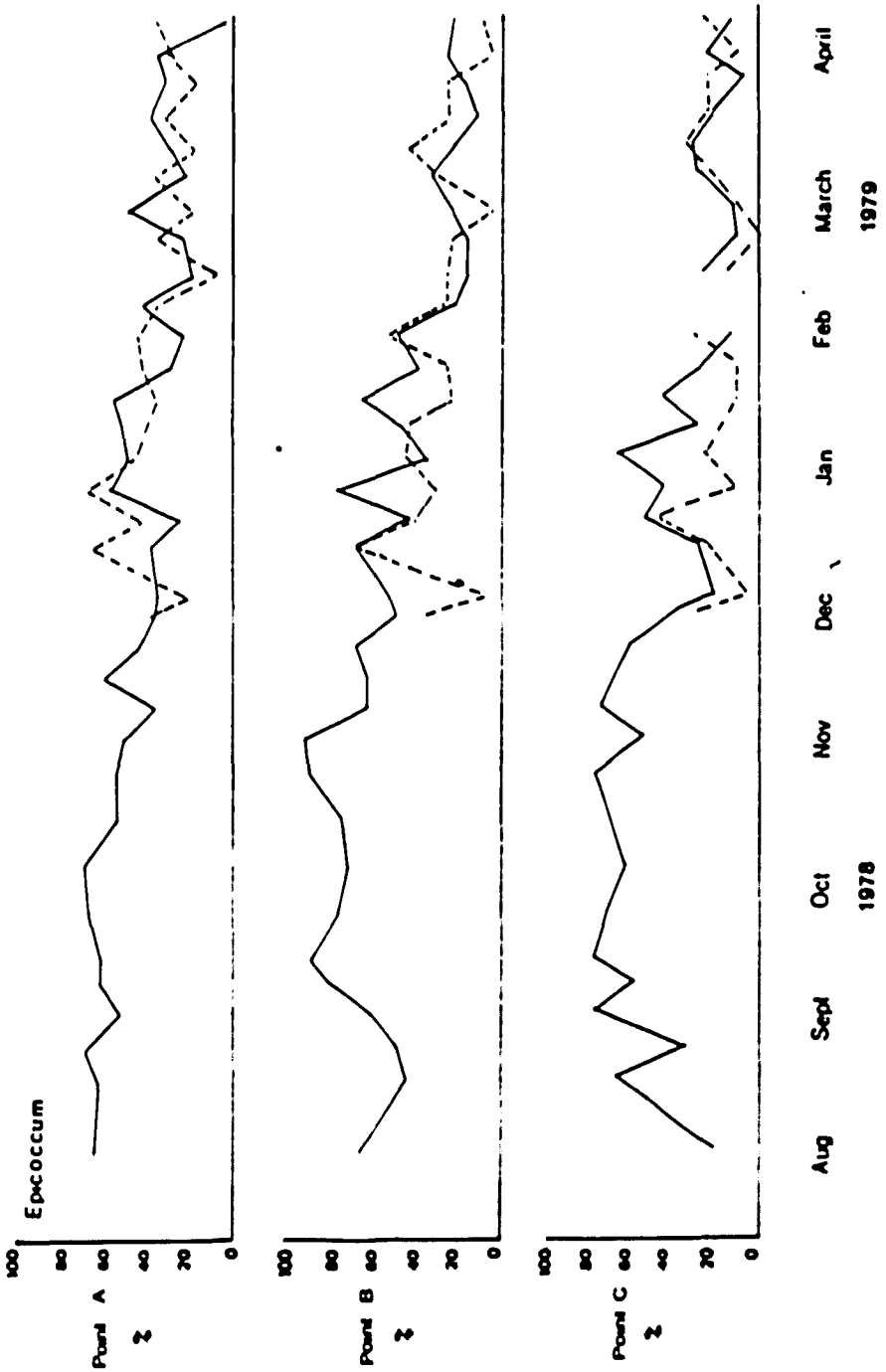

Epicoccum
Pond A
Pond B
Pond C
%
100
80
60
40
20
0
Aug
Sept
Oct
Nov
Dec
Jan
Feb
March
April
1978
1979

Fig. 13

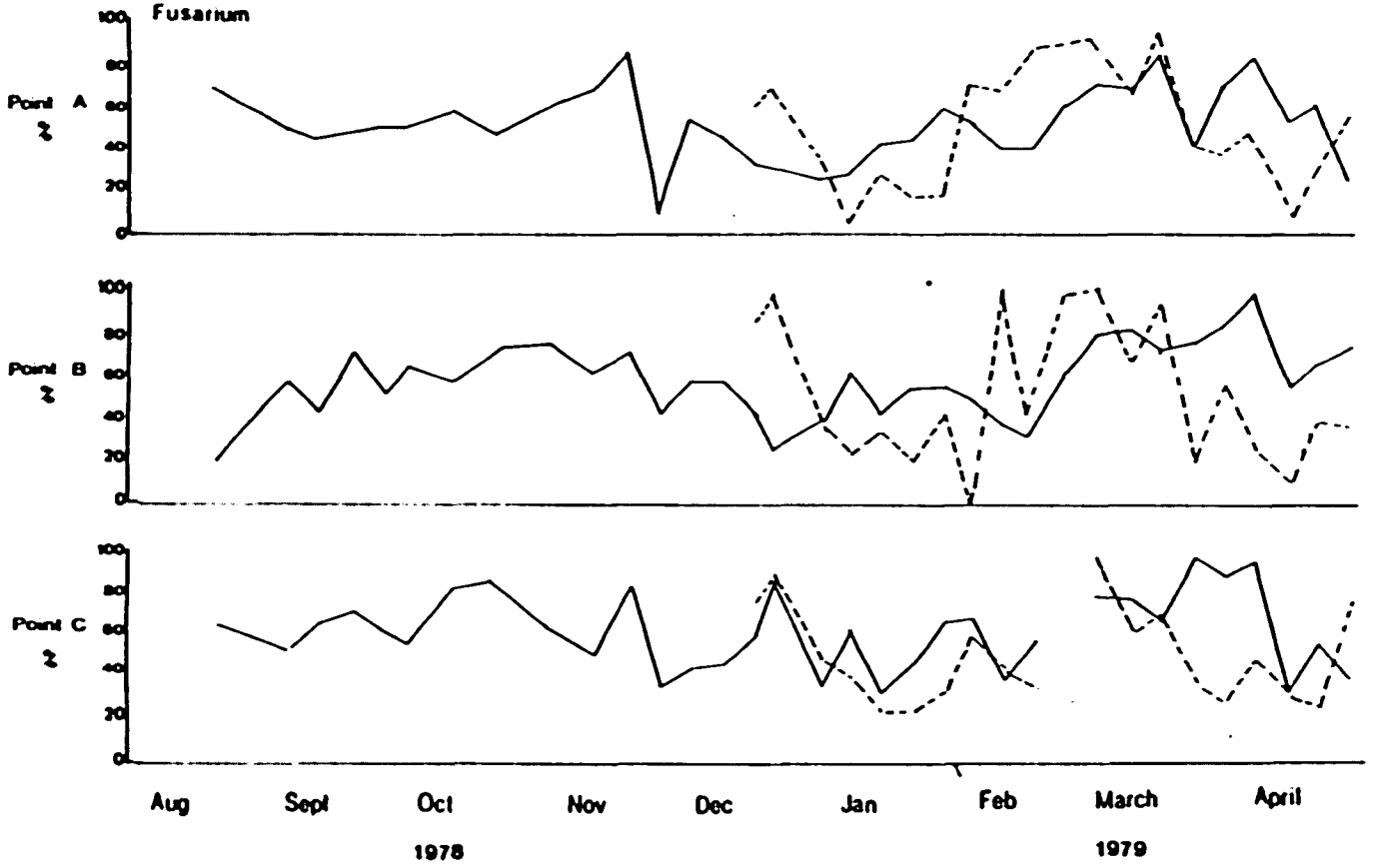

Fusarium
Point A
%
Point B
%
Point C
%
100
80
60
40
20
0
Aug
Sept
Oct
Nov
Dec
Jan
Feb
March
April
1978
1979

Fig. 14

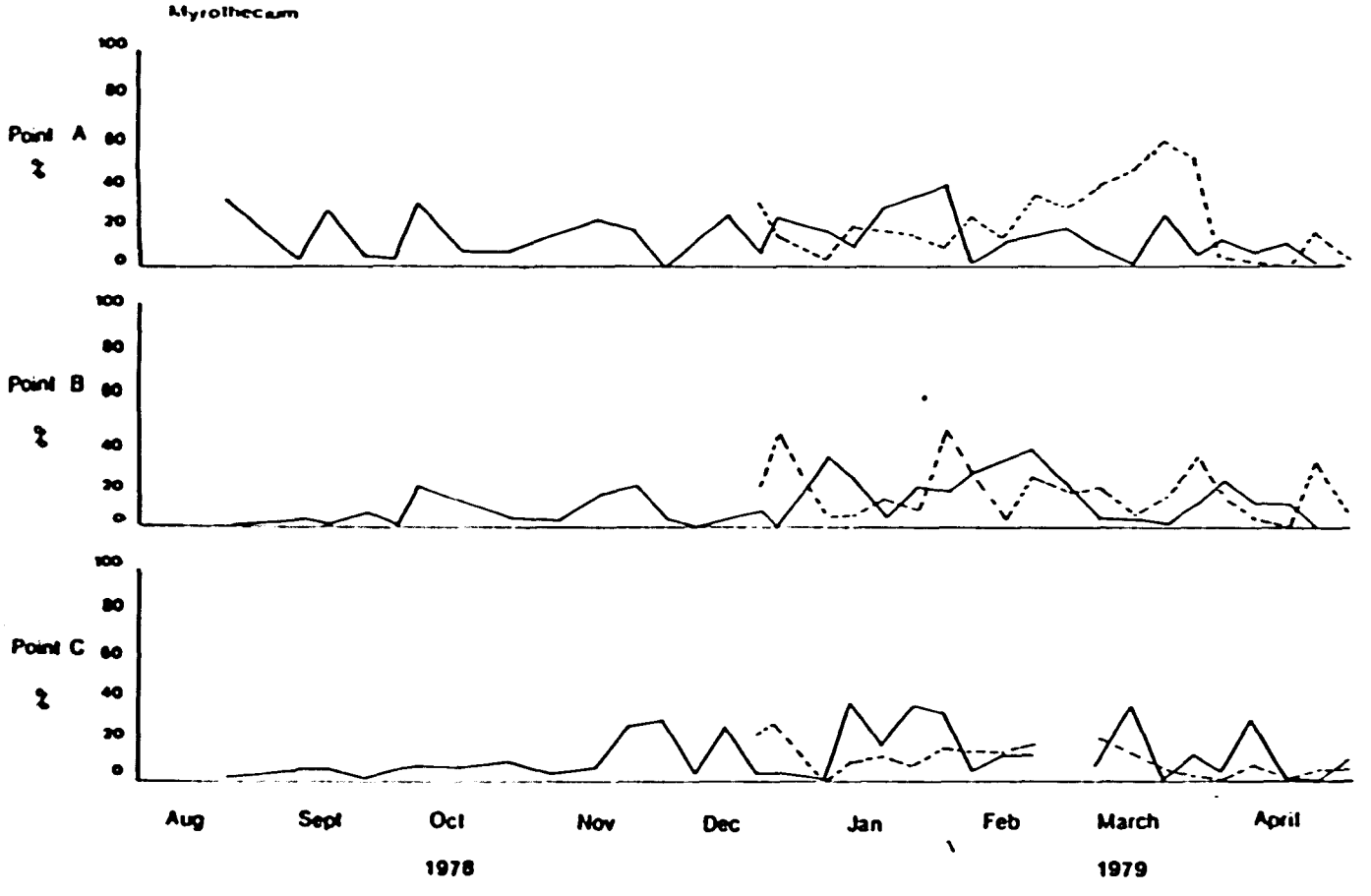

Myrothecium
Point A
%
Point B
%
Point C
%
100
80
60
40
20
0
Aug
Sept
Oct
Nov
Dec
Jan
Feb
March
April
1978
1979

Fig. 15

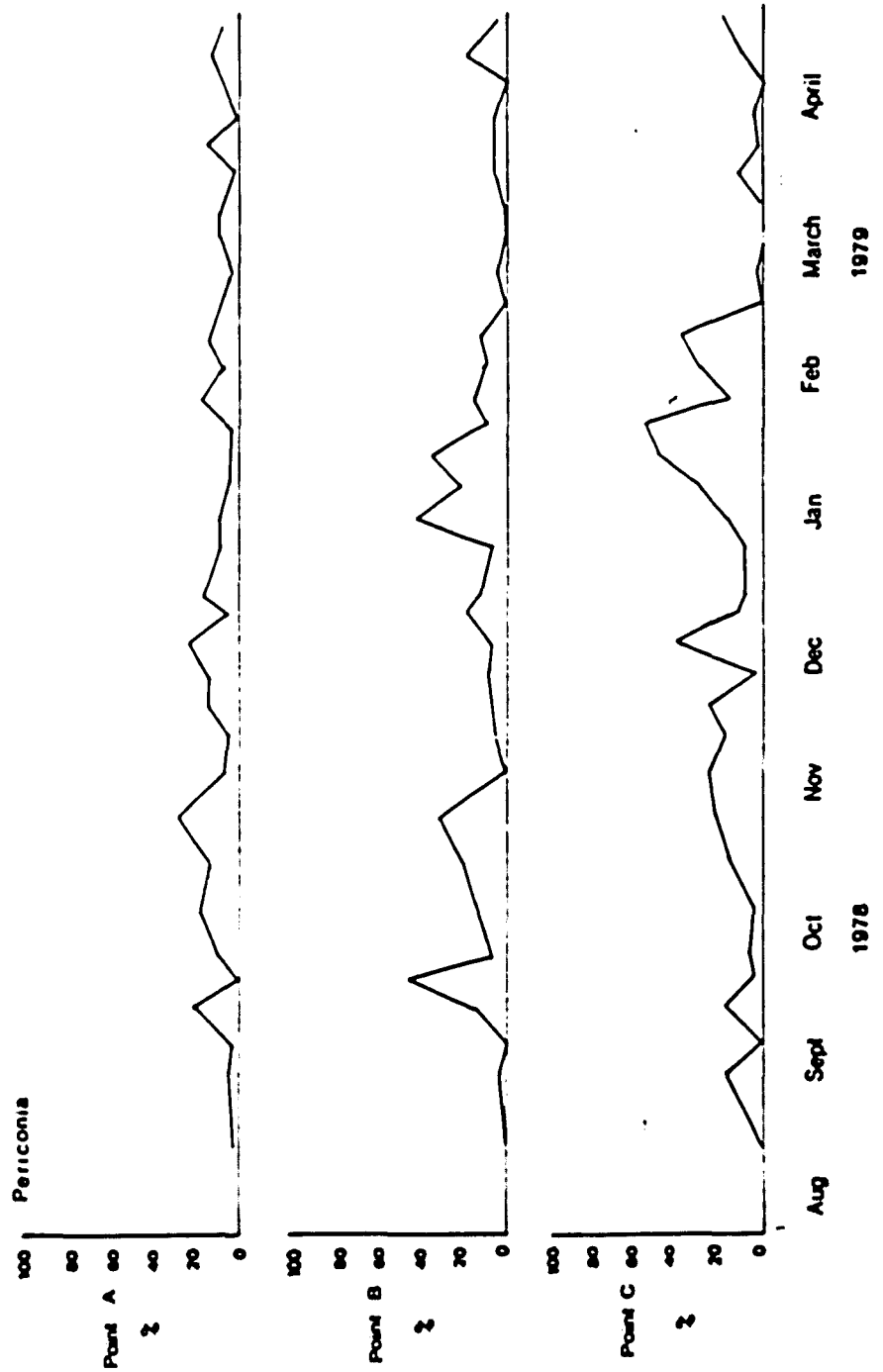

Perriconia
Point A
%
100
80
60
40
20
0
Point B
%
100
80
60
40
20
0
Point C
%
100
80
60
40
20
0
Aug
Sept
Oct
1978
Nov
Dec
Jan
Feb
March
1979
April

Fig. 16

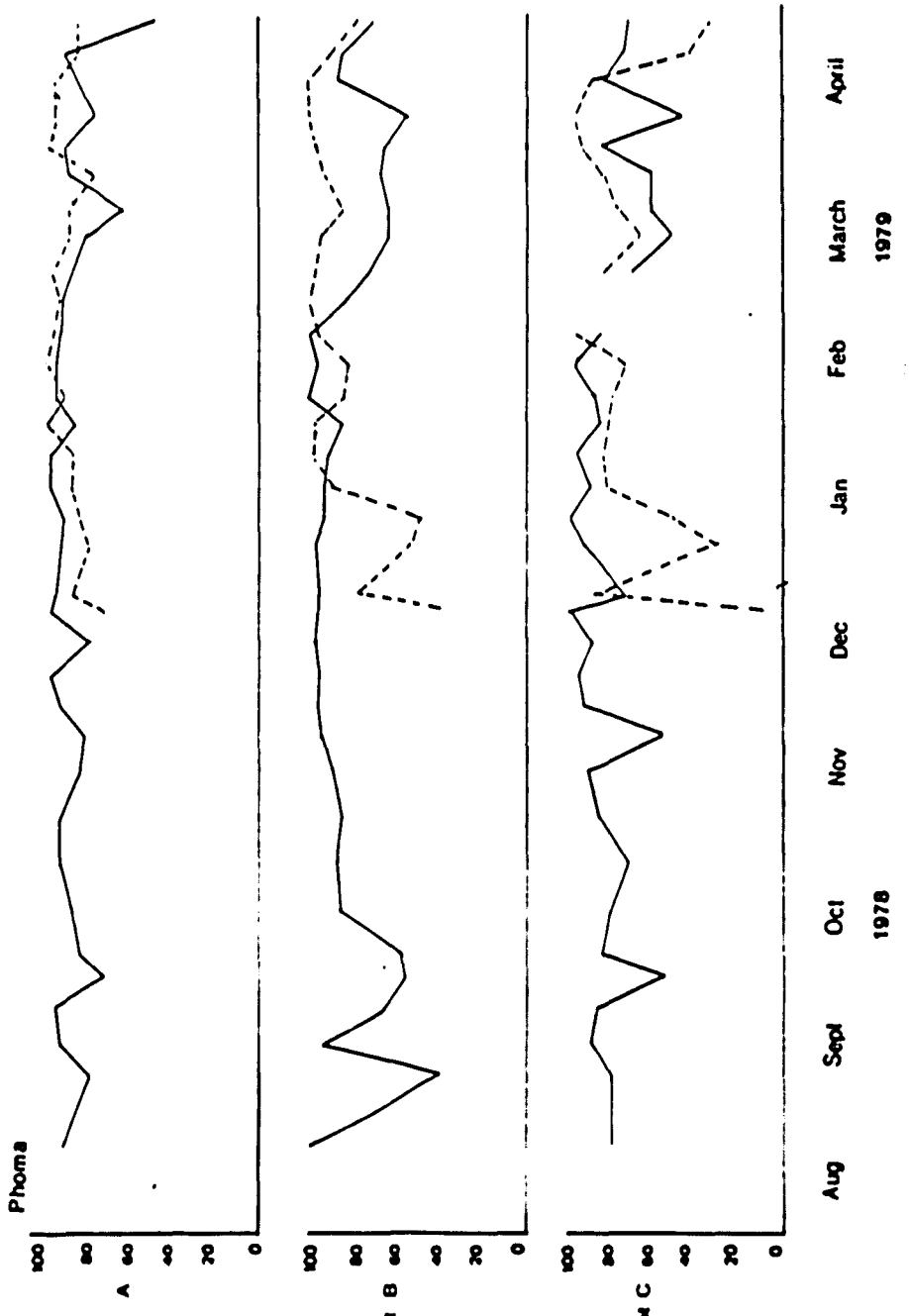
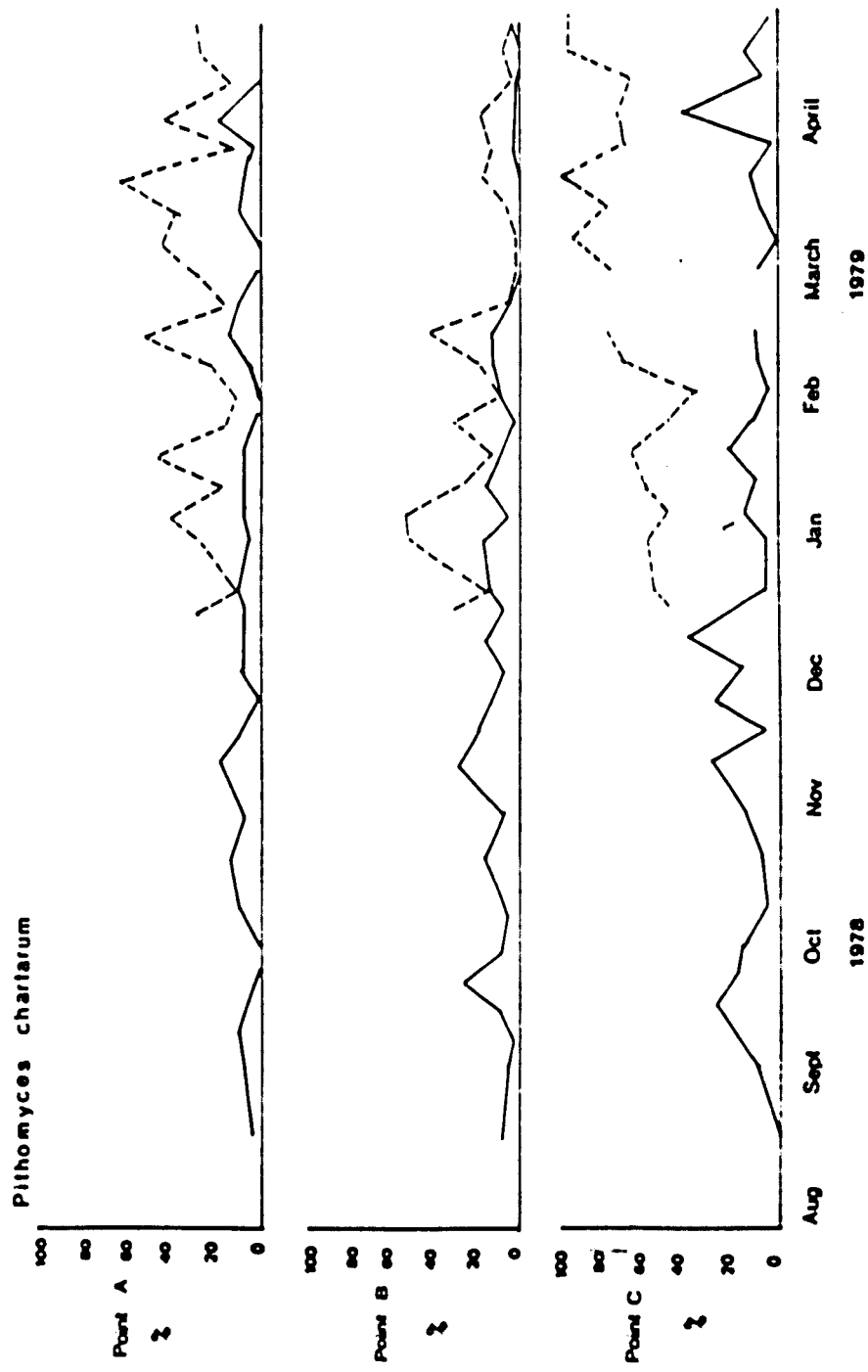

Phoma
Point A
%
100
80
60
40
20
0
Point B
%
100
80
60
40
20
0
Point C
%
100
80
60
40
20
0
Aug
Sept
Oct
Nov
Dec
Jan
Feb
March
April
1978
1979

Fig. 17


Pithomyces charlarum
Point A
Point B
Point C
%
100
80
60
40
20
0
Aug
Sept
Oct
Nov
Dec
Jan
Feb
March
April
1978
1979

Figs. 18 - 21

Incidence of selected fungi in Camps A & B from December 1979 to March 1980 as a percentage of the nine samples

LEGEND

Ci Cynodon incompletus
El Eragrostis lehmanniana
Fm Felicia muricata
Gp Galenia procumbens
Gs Galenia sarcophylla
Lc Lycium cinereum
Tt Tribulus terrestris
Litter

Fig. 18

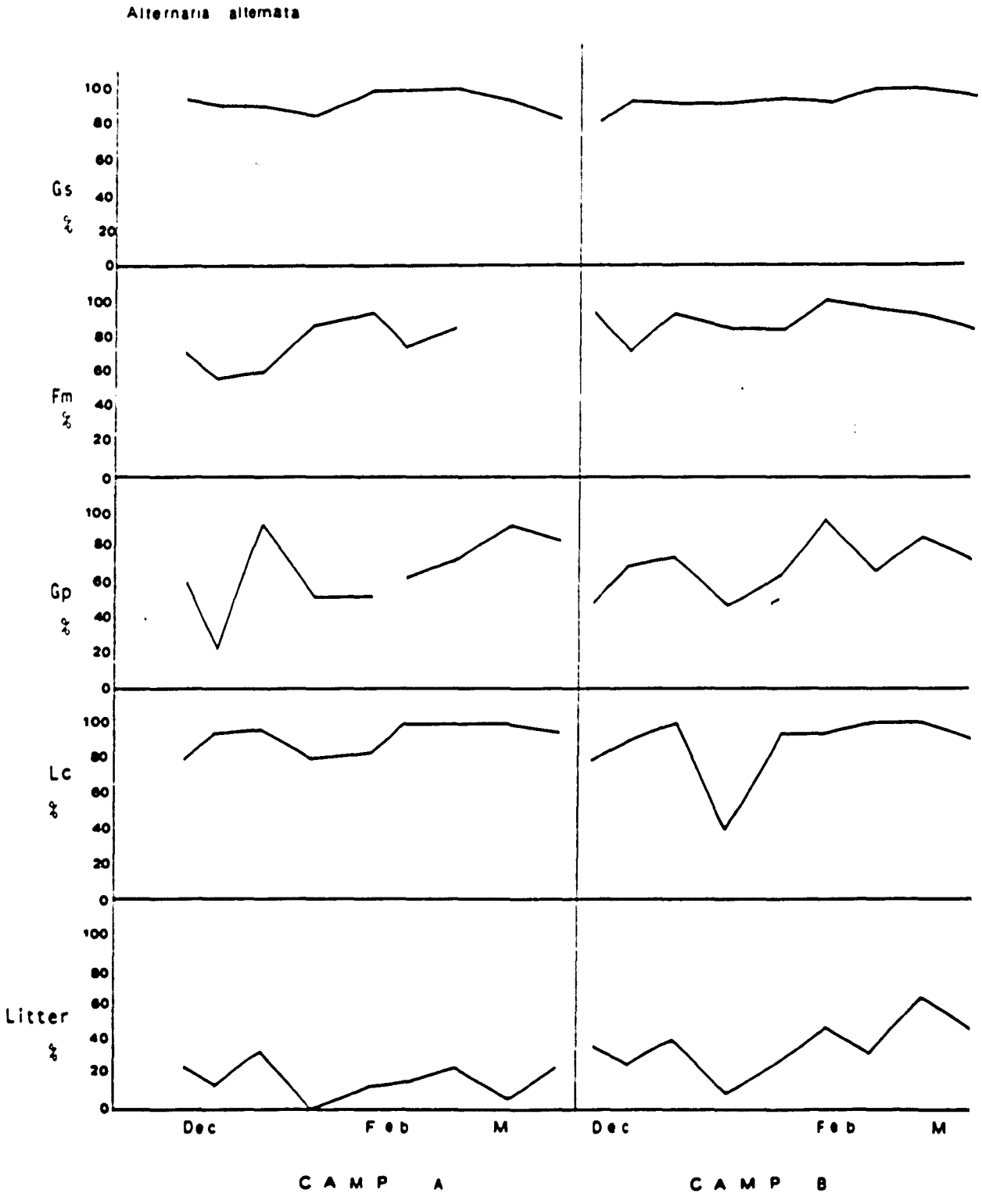

Alternaria alternata
Gs %
Fm %
Gp %
Lc %
Litter %
100
80
60
40
20
0
Dec
Feb
M
CAMP A
CAMP B

Fig. 19

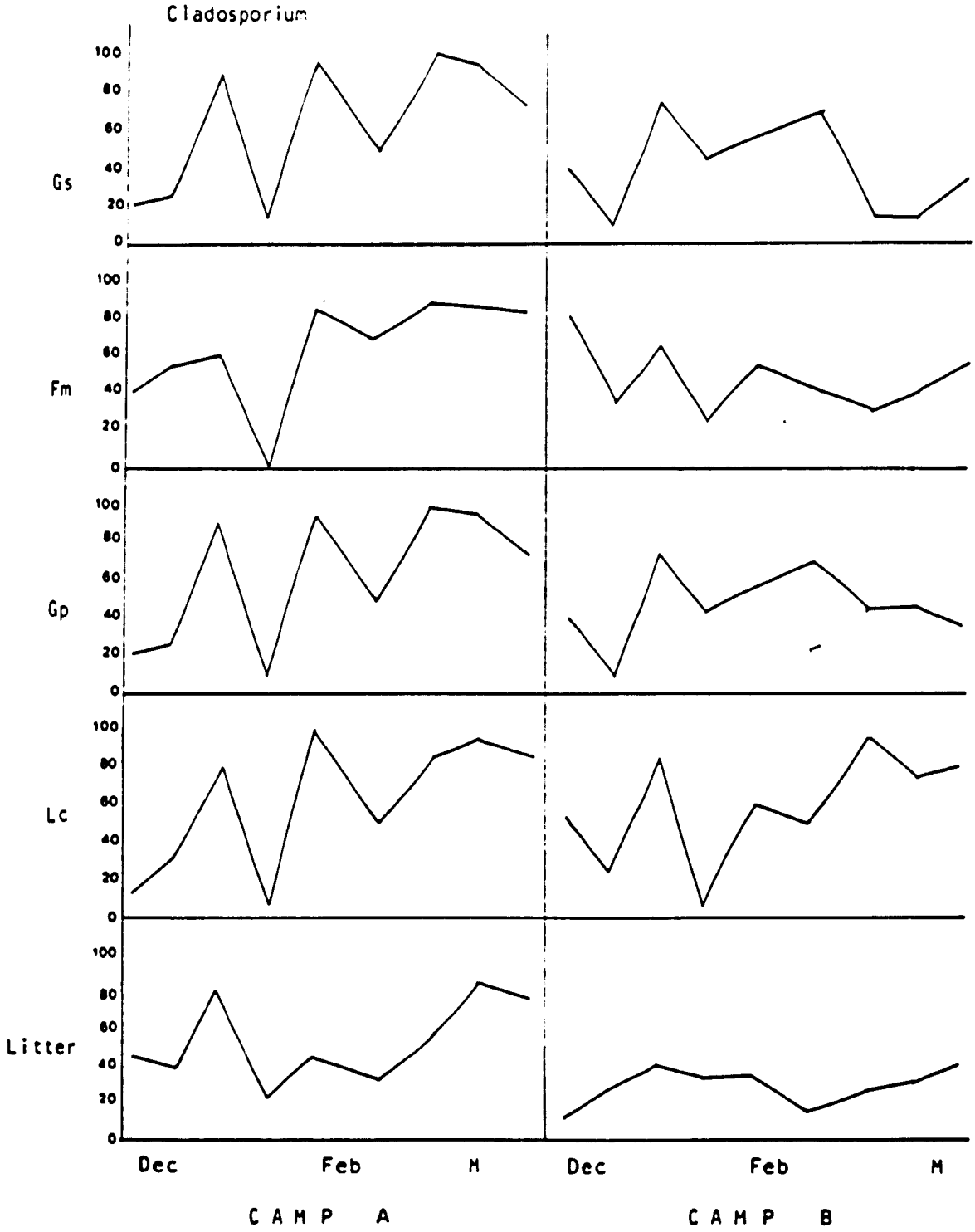

Cladosporium
Gs
Fm
Gp
Lc
Litter
100
80
60
40
20
0
Dec
Feb
M
CAMP A
CAMP B

Fig. 20

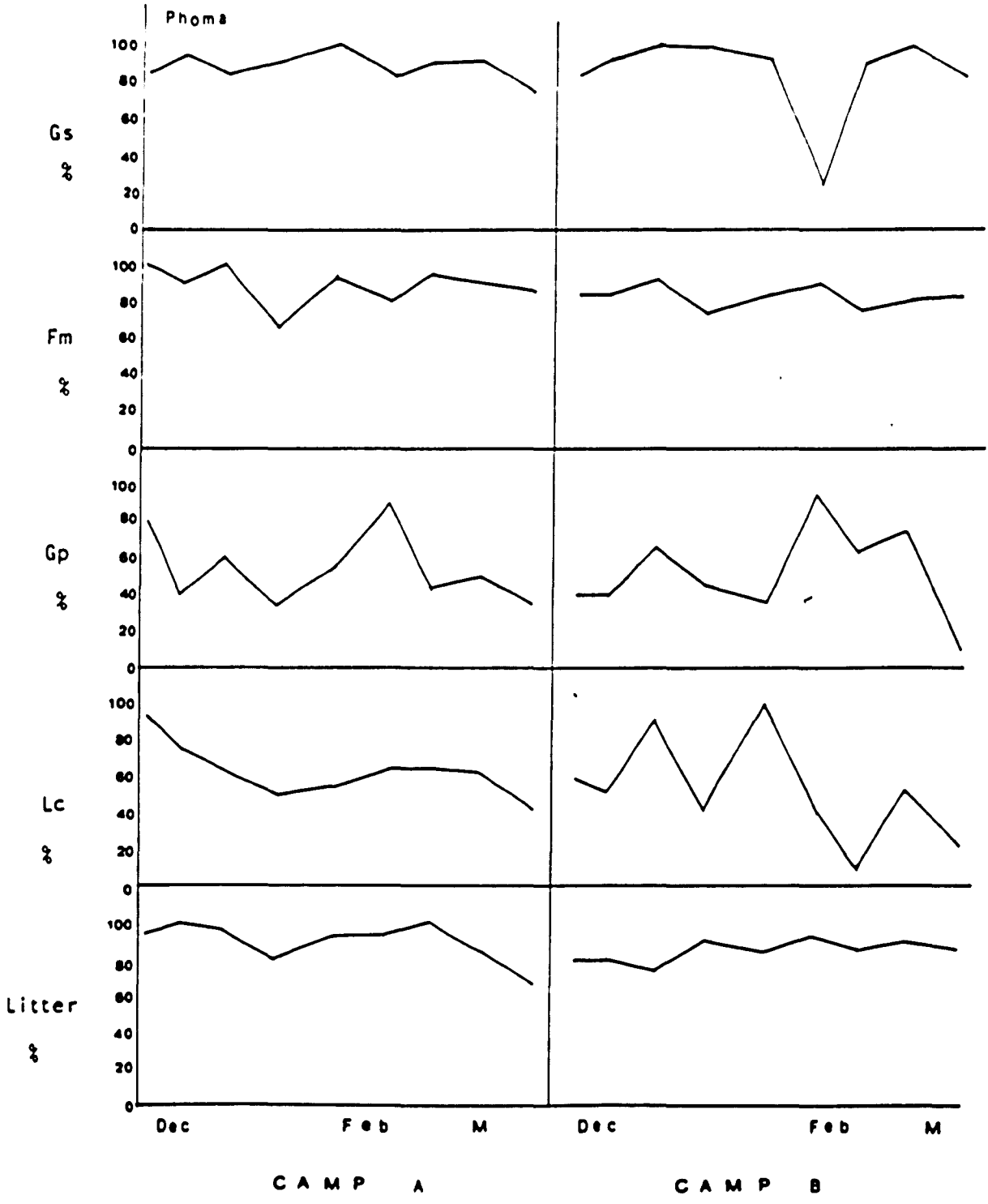
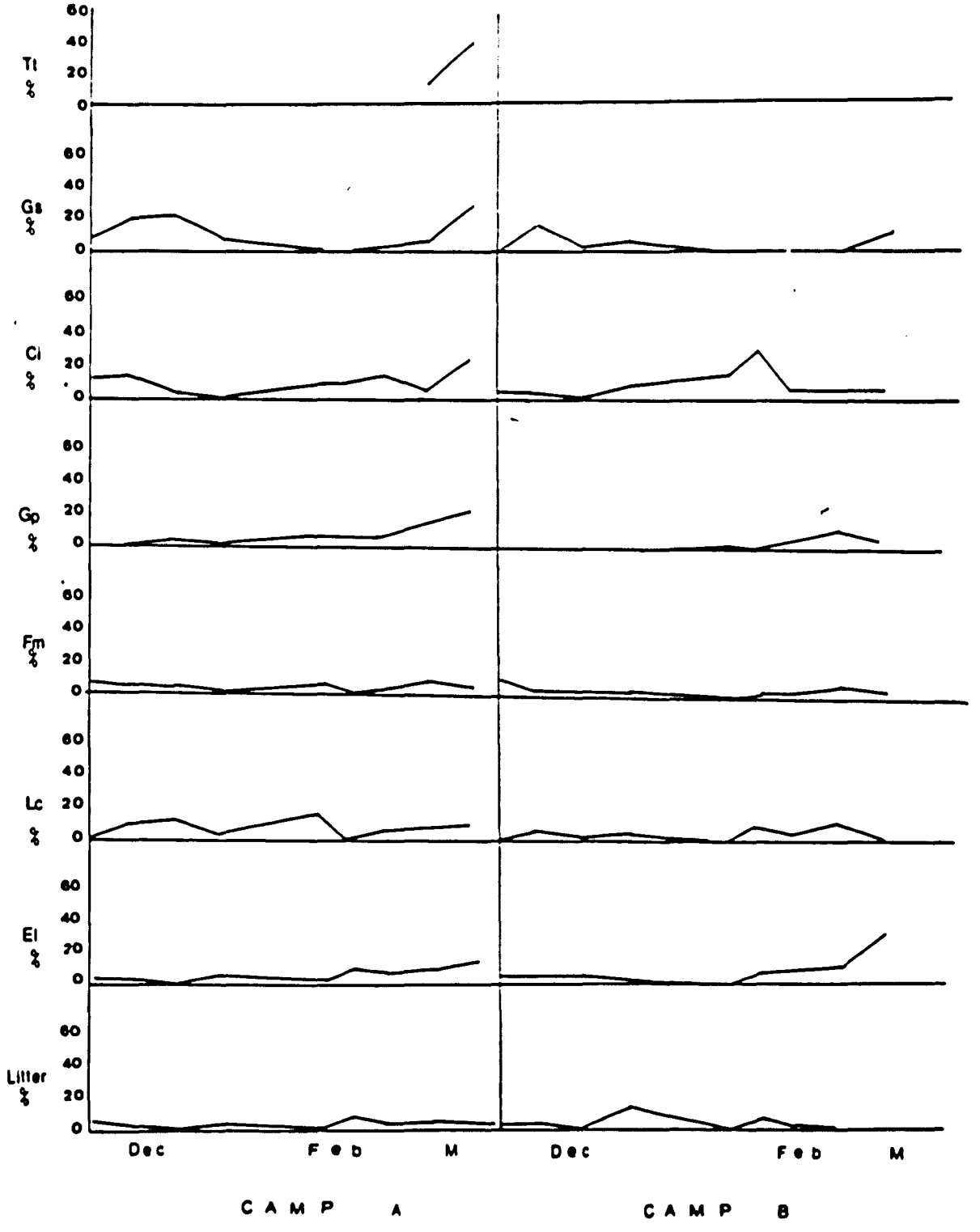


Fig. 21

Pithomyces chartarum



Figs. 22 - 35

Incidence of selected fungi in Camps A & B from October 1980 to September 1981 expressed as a percentage

LEGEND

Cl Cynodon incompletus
El Eragrostis lehmanniana
Fm Felicia muricata
Gp Galenia procumbens
Gs Galenia sarcophylla
Lc Lycium cinereum
Tt Tribulus terrestris

and the litter fraction

Fig. 22 - A

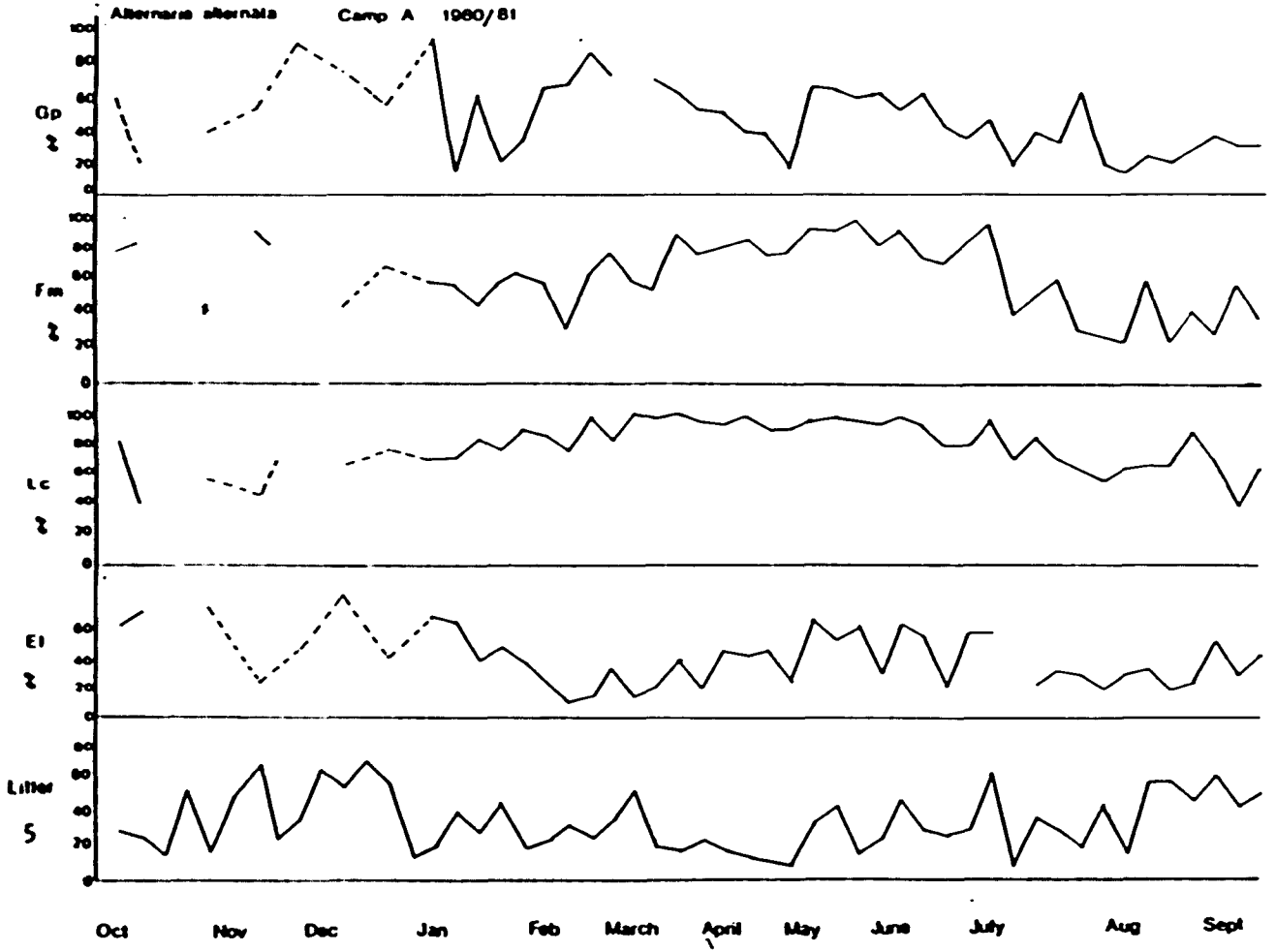
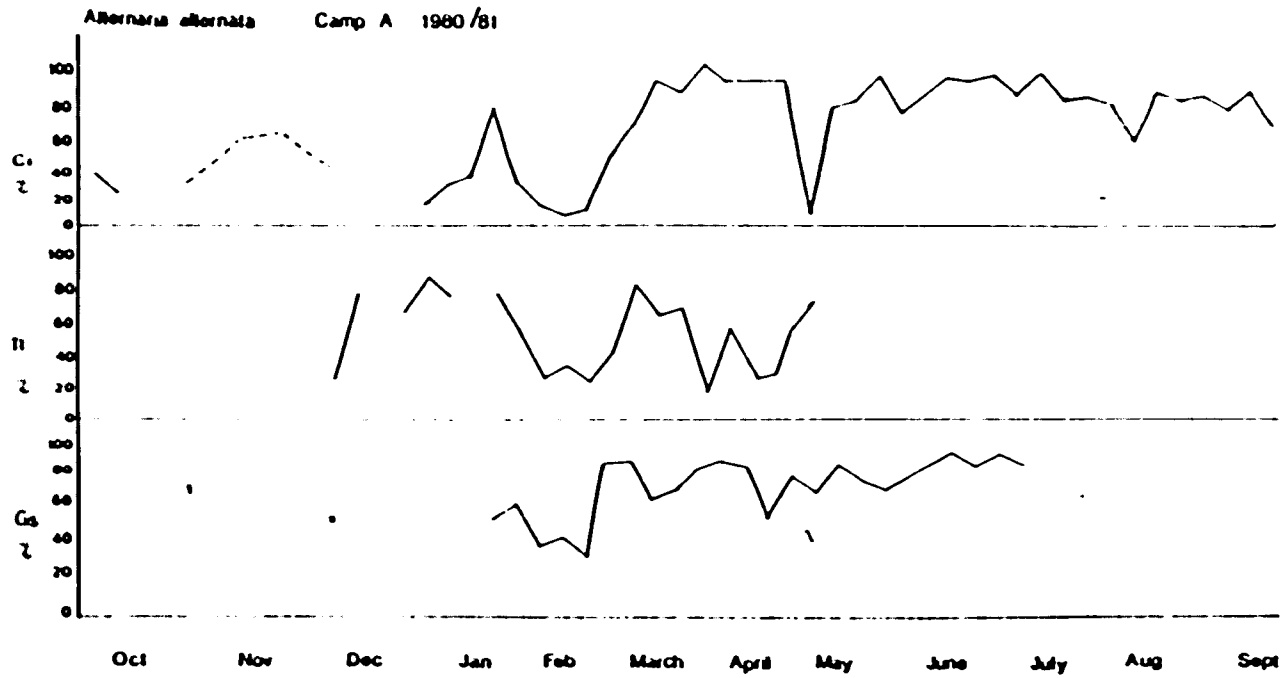

Alternaria alternata
Camp A 1980/81
Gp
%
Fm
%
Lc
%
El
%
Litter
S
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

Fig. 22 - A


Alternaria alternata Camp A 1980/81
100
80
60
40
20
0
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

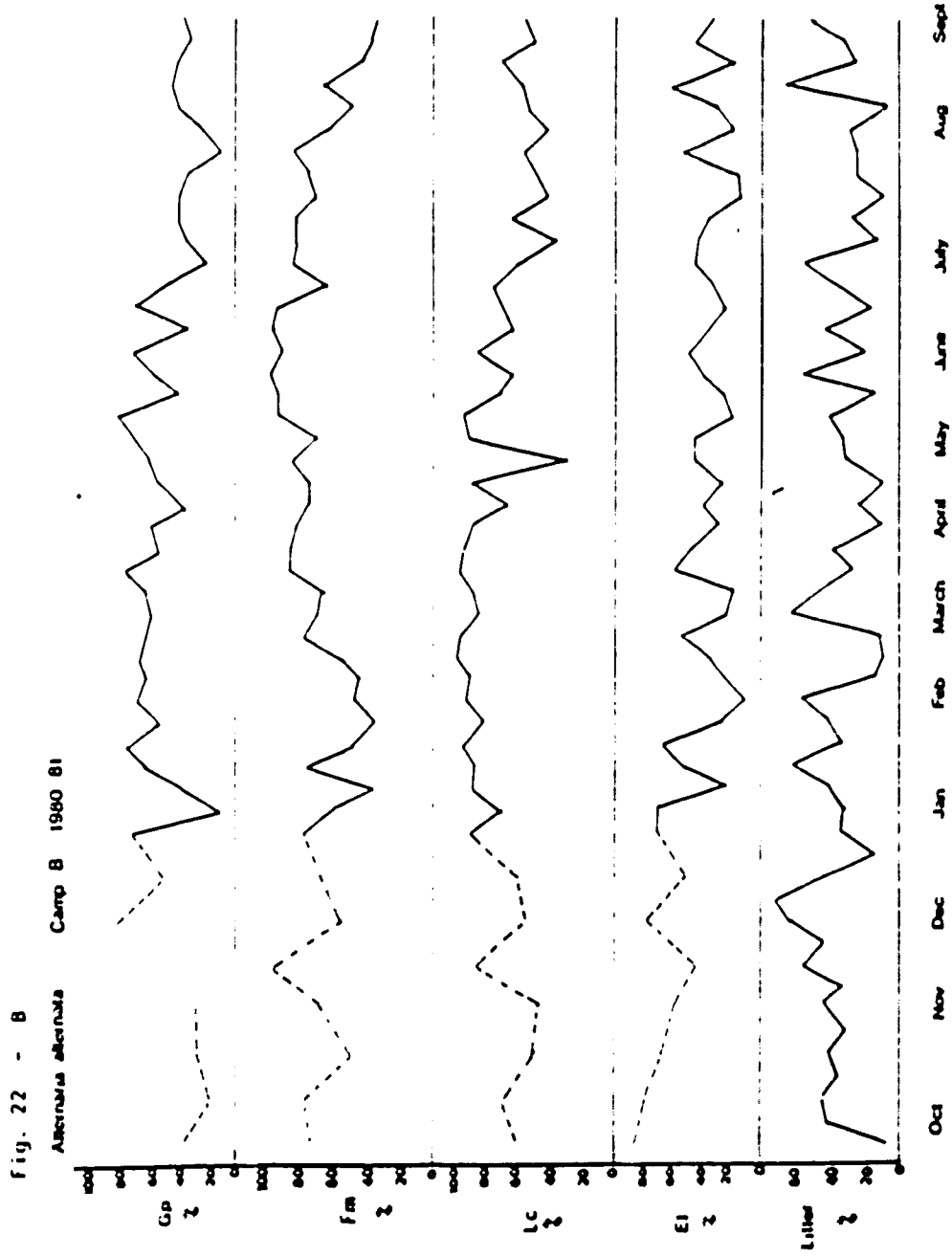


Fig. 22 - B
Alternaria alternata Camp B 1980 81
Gp
Fm
Lc
El
Lilac
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

Fig. 22 - B

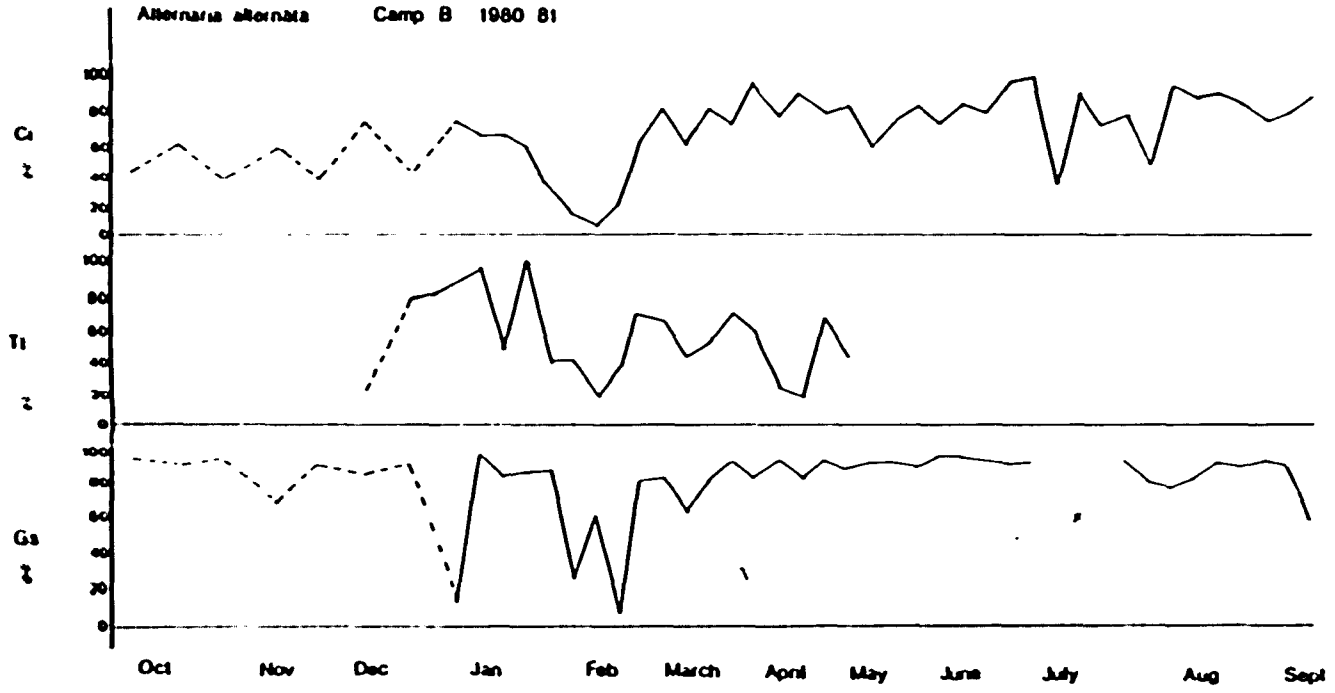
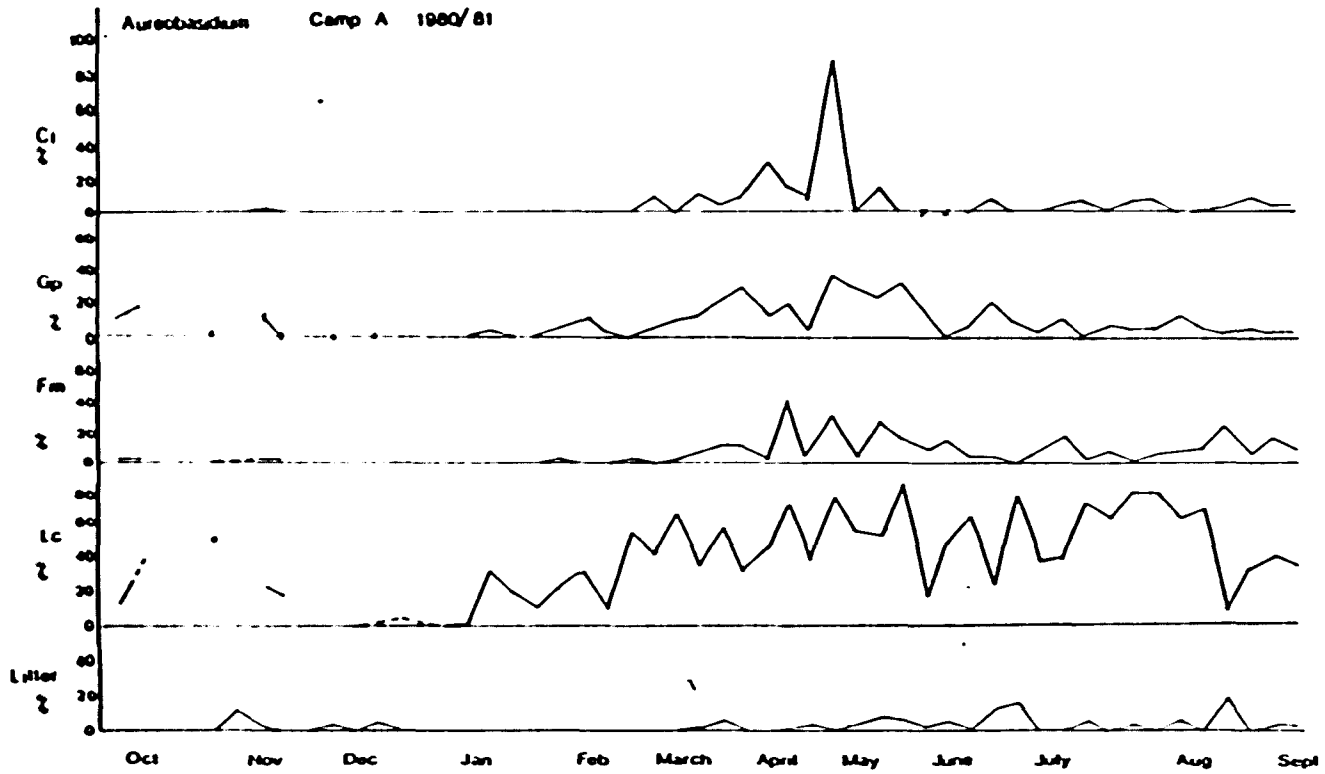

Alternaria alternata Camp B 1980 81
Ci
%
Ti
%
Gs
%
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

Fig. 23 - A


Aureobasidium
Camp A 1980/81
Cl %
Gp %
Fm %
Lc %
Litter %
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

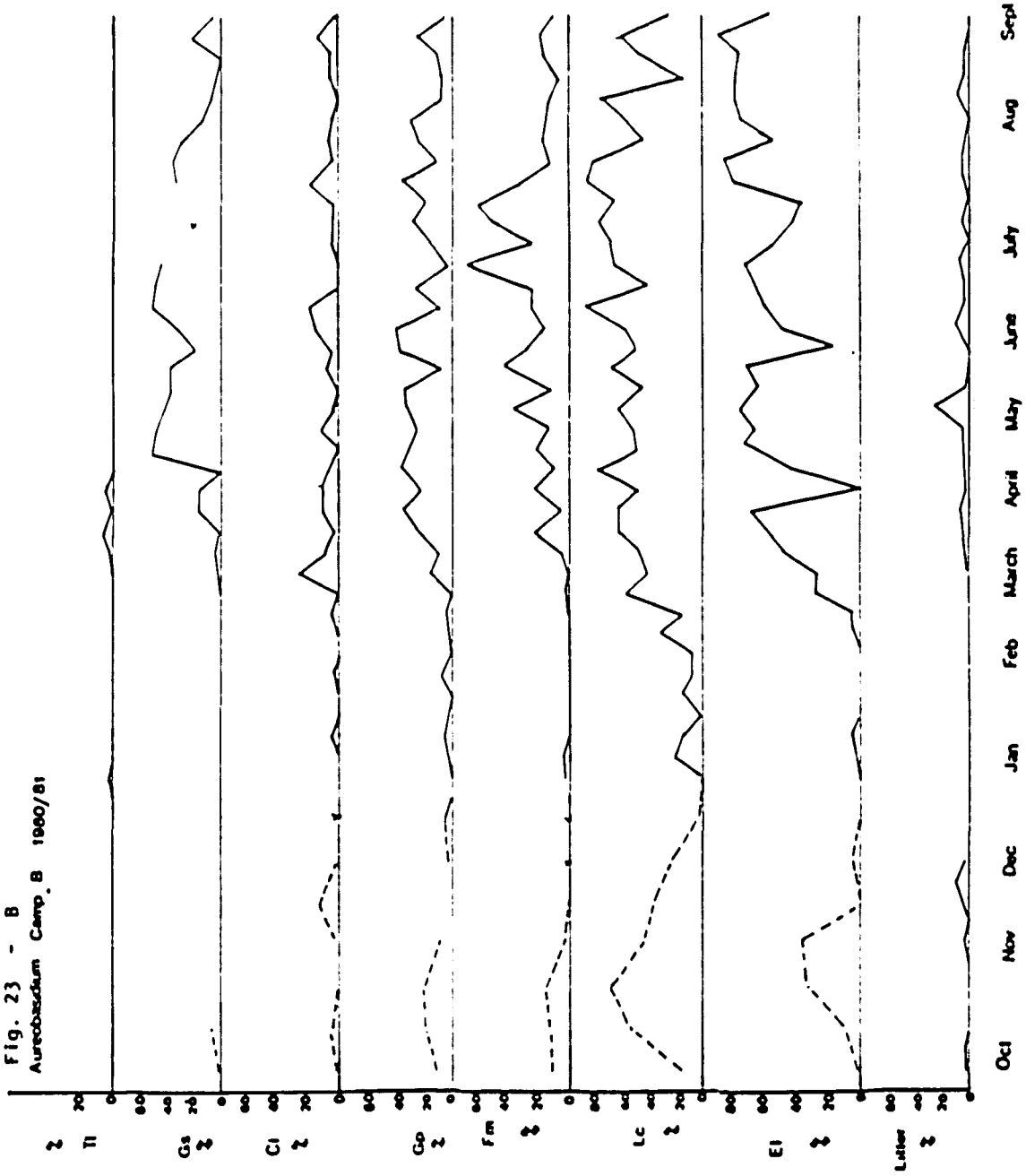


Fig. 23 - B
Aurecobasidium Camp. B 1980/81
Tl
Gs
Cl
Cp
Fm
Lc
El
Litter
%
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

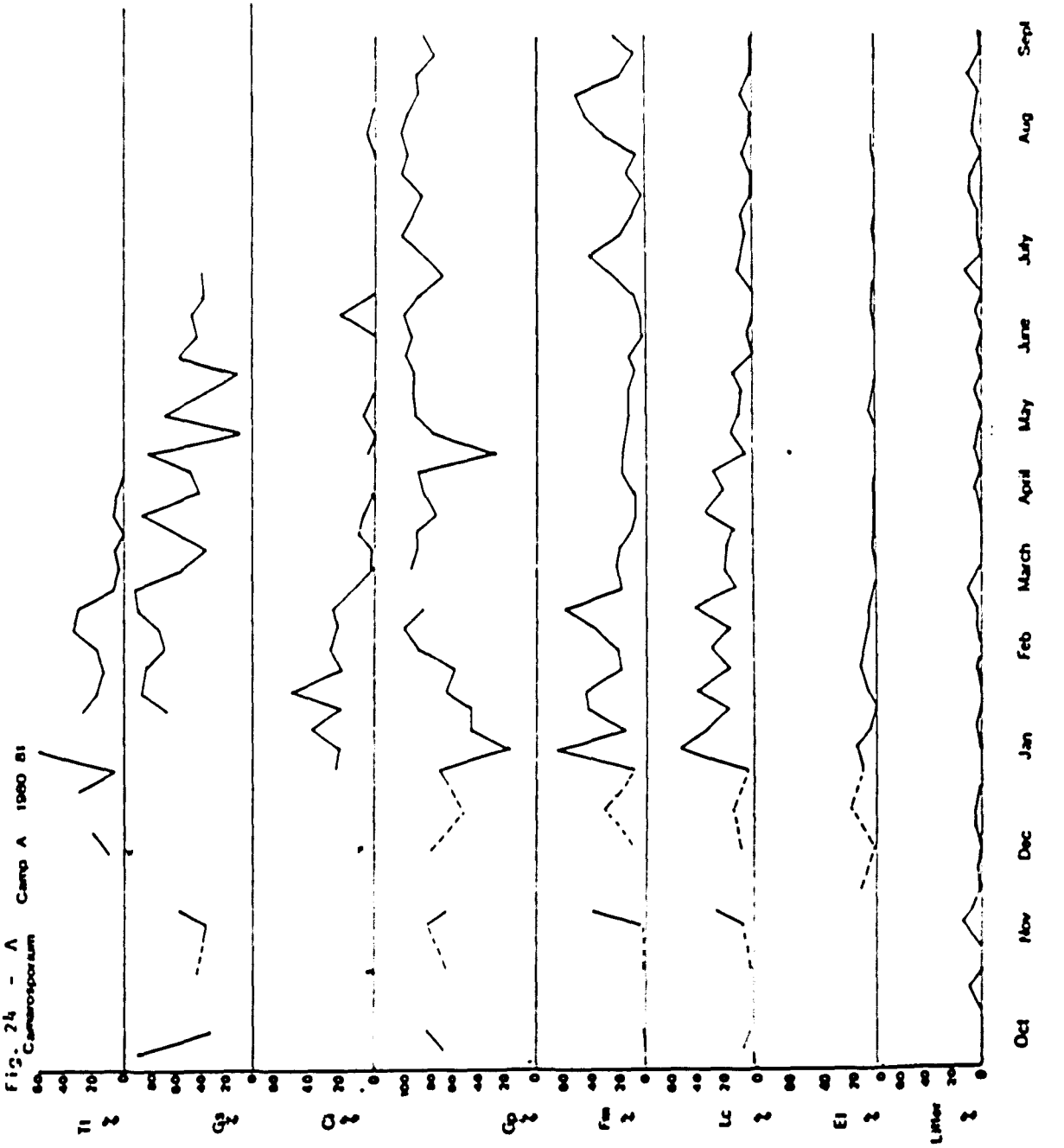


Fig. 24 - A
Camarosporium
Camp A 1980 81
Tl %
Gs %
Cs %
Gp %
Fm %
Lc %
El %
Litter %
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

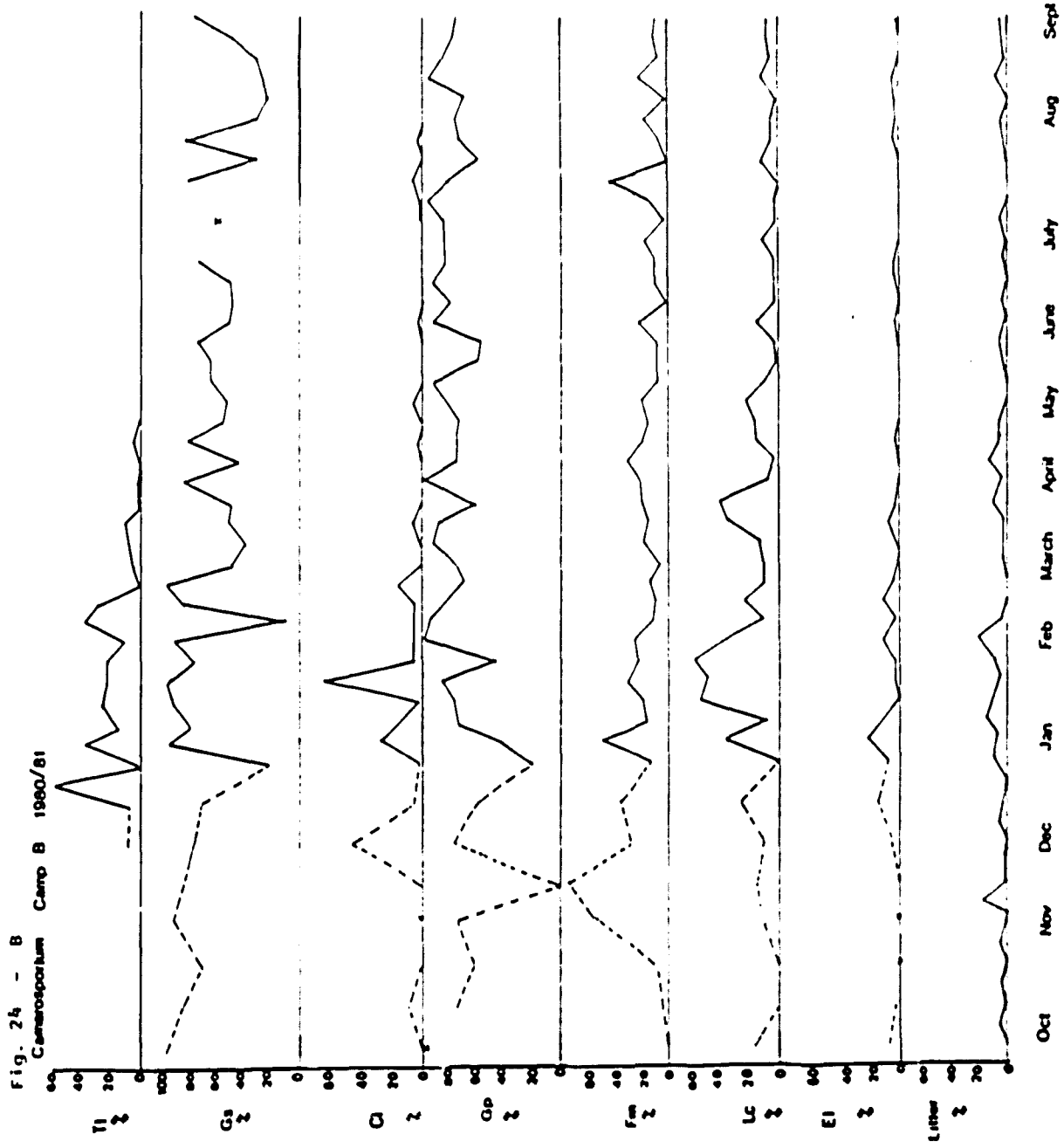


Fig. 24 - B
Cannatosporium Camp B 1980/81
Tl
Gs
Cl
Op
Fm
Lc
El
Litter
%
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

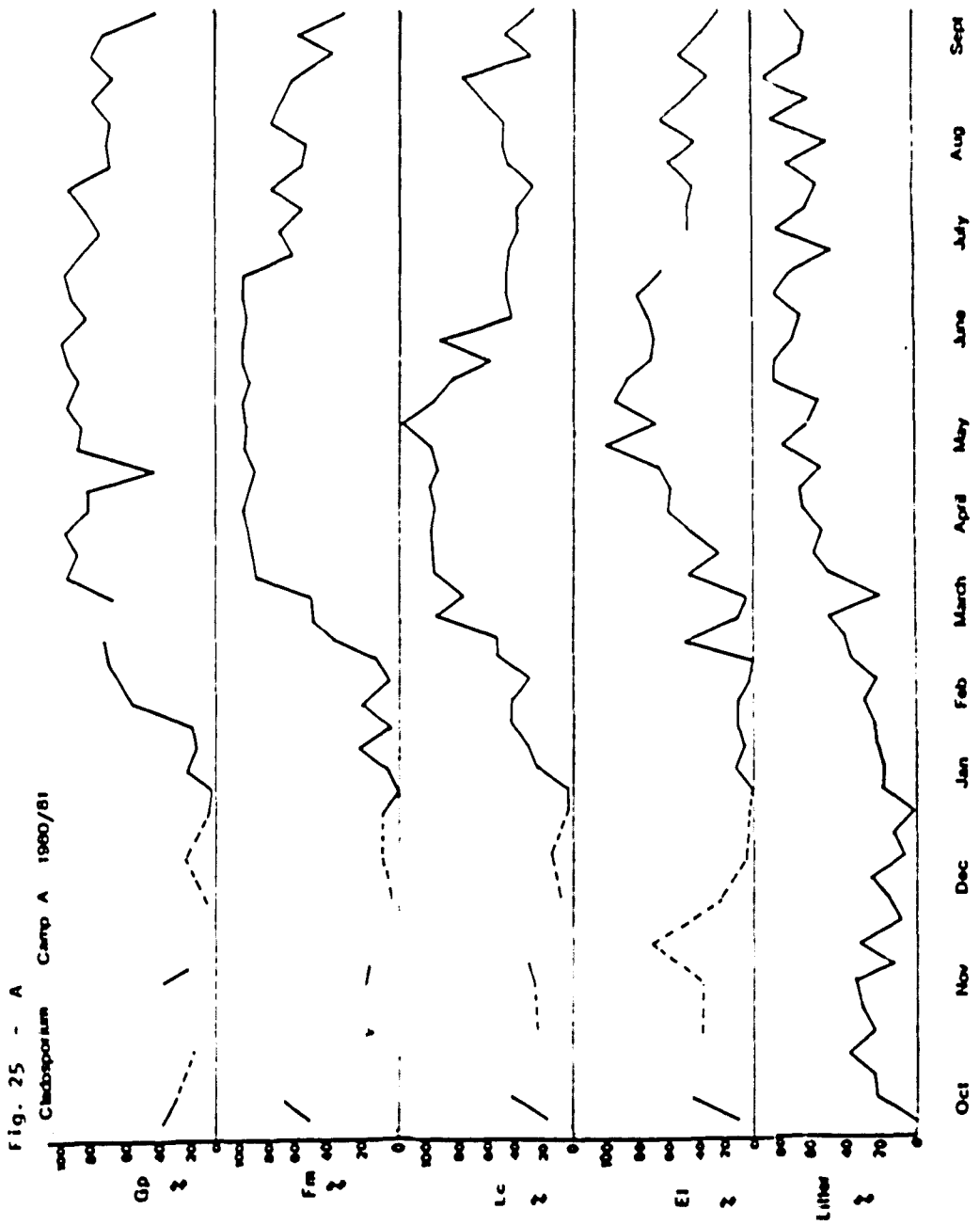


Fig. 25 - A
Cladosporium Camp A 1980/81
Op
Fm
Lc
El
Litter
%
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

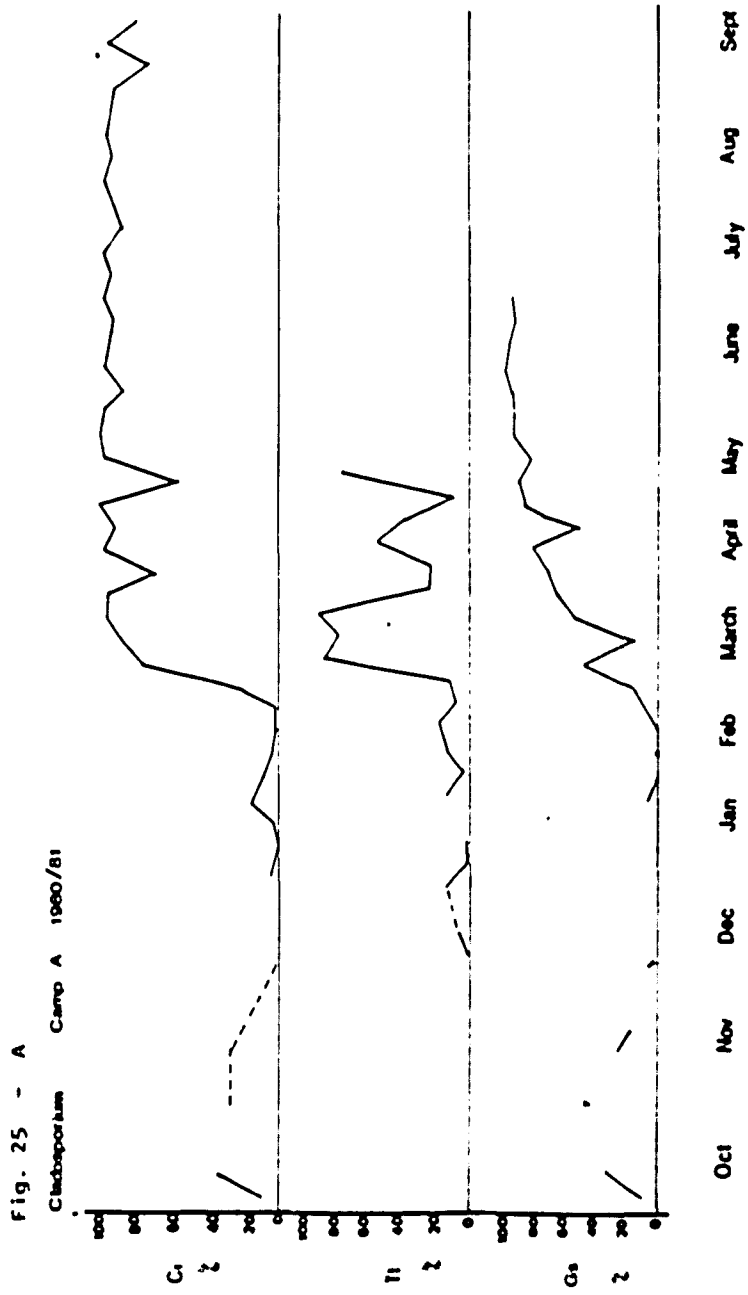


Fig. 25 - A
Cladosporium Camp A 1980/81
Cl %
Tl %
Cs %
100
80
60
40
20
0
Oct Nov Dec Jan Feb March April May June July Aug Sept

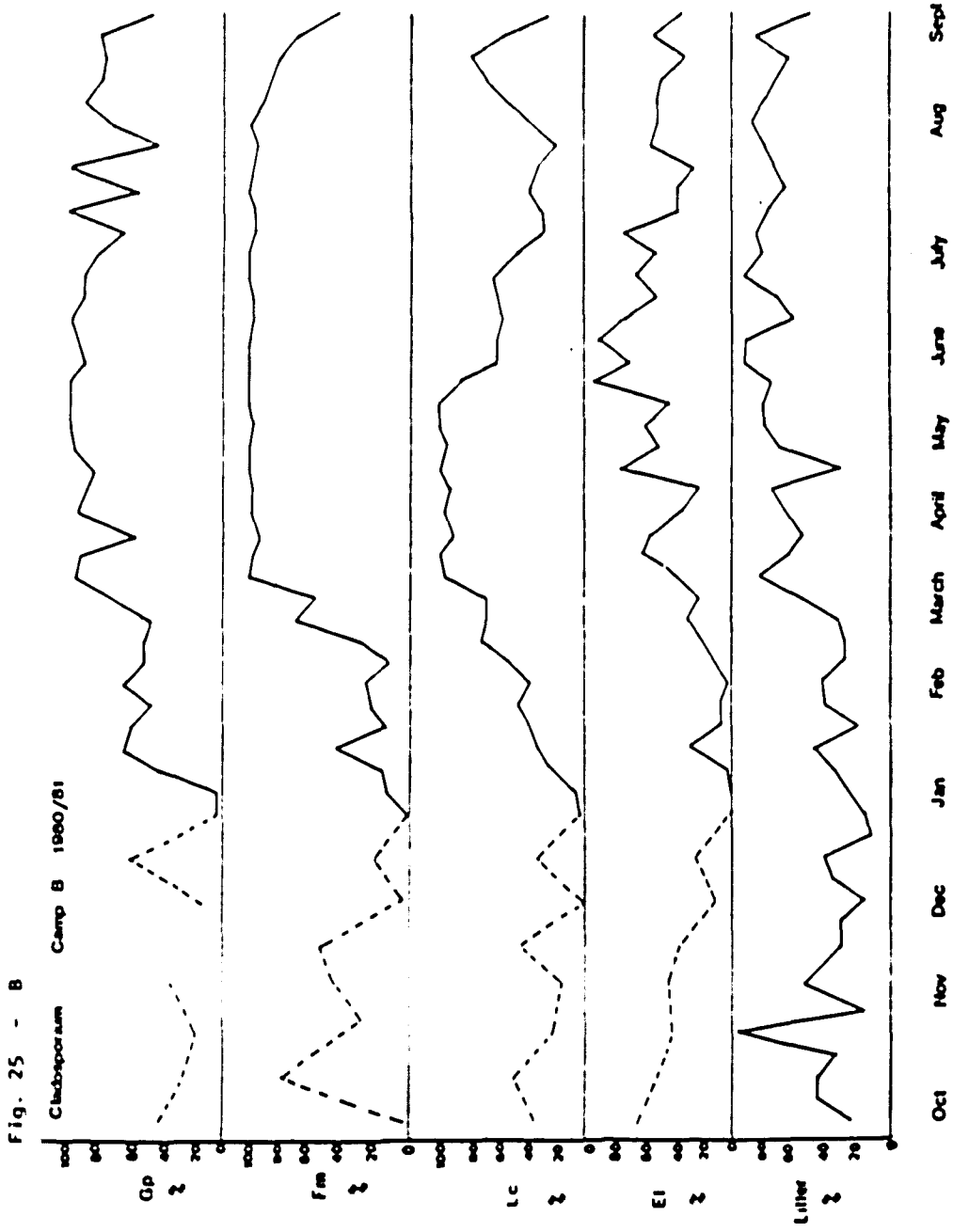


Fig. 25 - B
Cladosporium Camp B 1980/81
Gp
Fm
Lc
El
Litter
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

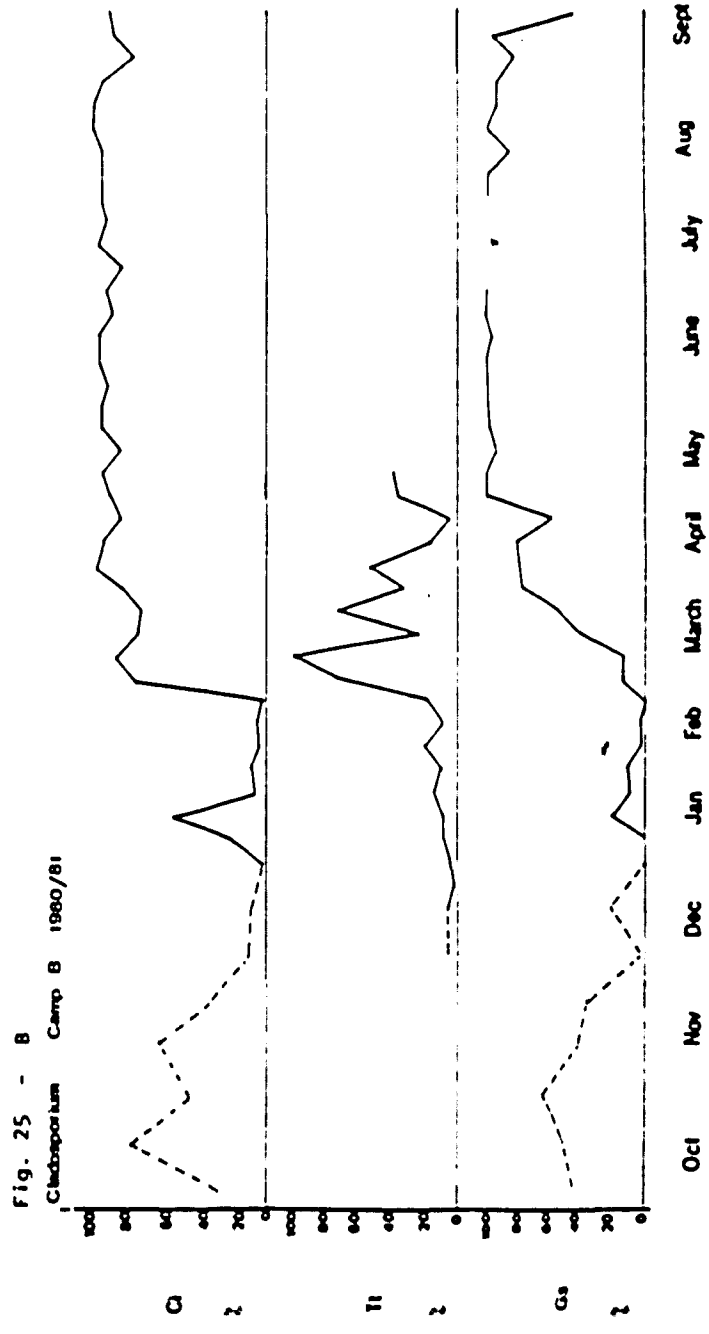


Fig. 25 - B
Cladosporium Camp B 1980/81
Cl
Tl
Gs
%
100
80
60
40
20
0
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

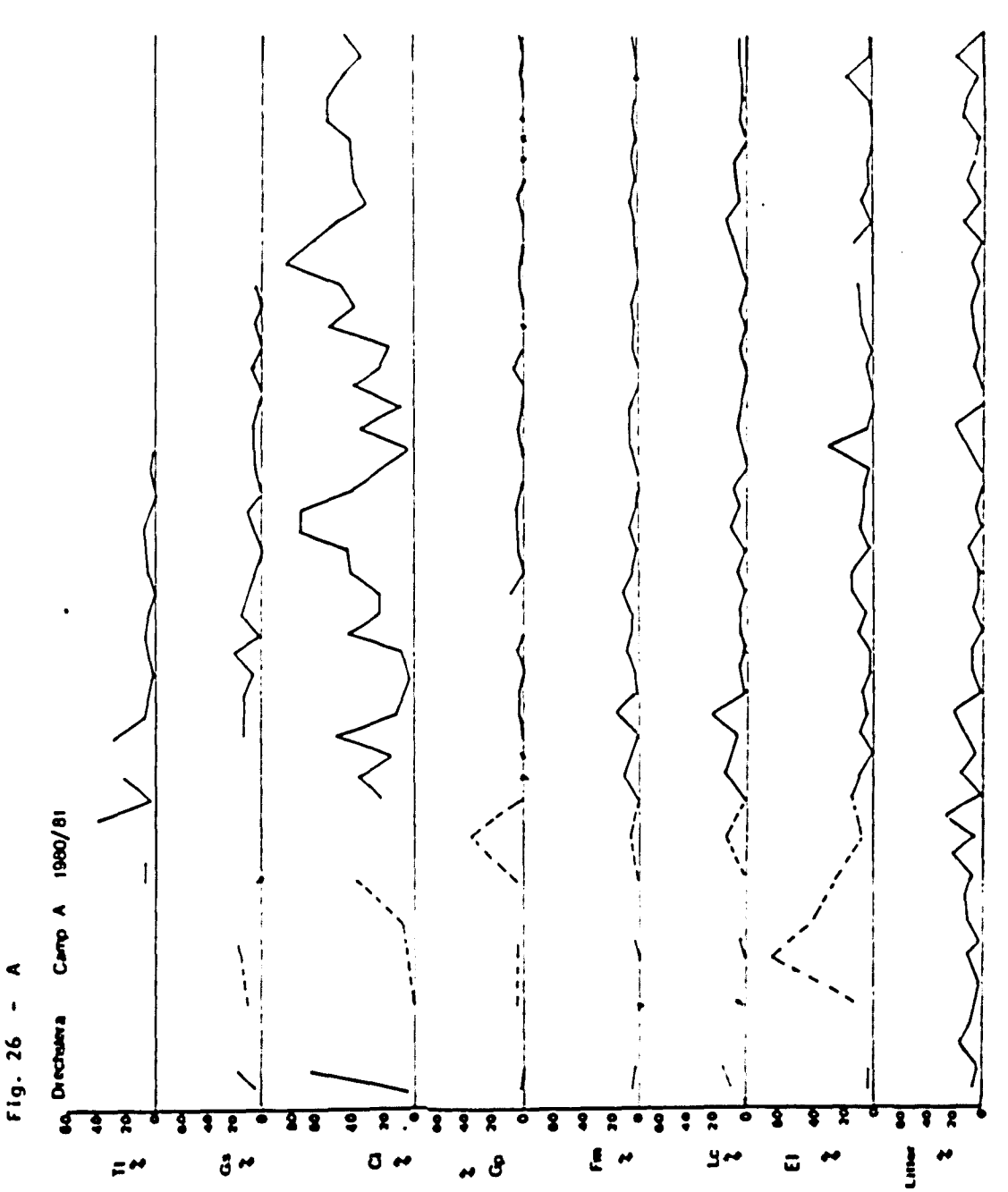


Fig. 26 - A
Drechslera Camp A 1980/81
Tl %
Cs %
Cl %
Gp %
Fm %
Lc %
El %
Litter %
Oct Nov Dec Jan Feb March April May June July Aug Sept

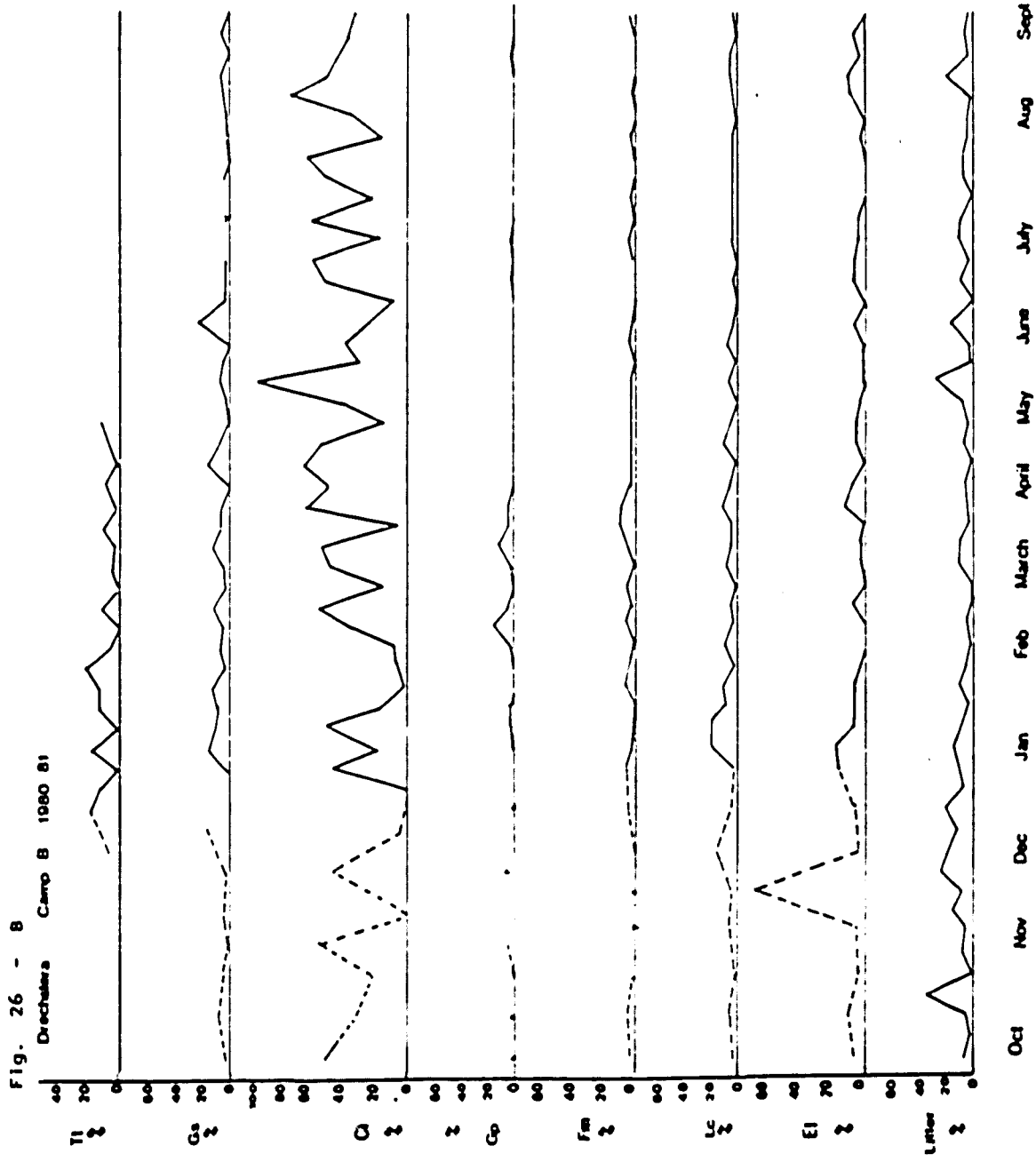


Fig. 26 - B
Drechslera Camp B 1980 81
Tl %
Ga %
Cl %
Gp %
Fm %
Le %
El %
Litter %
Oct Nov Dec Jan Feb March April May June July Aug Sept

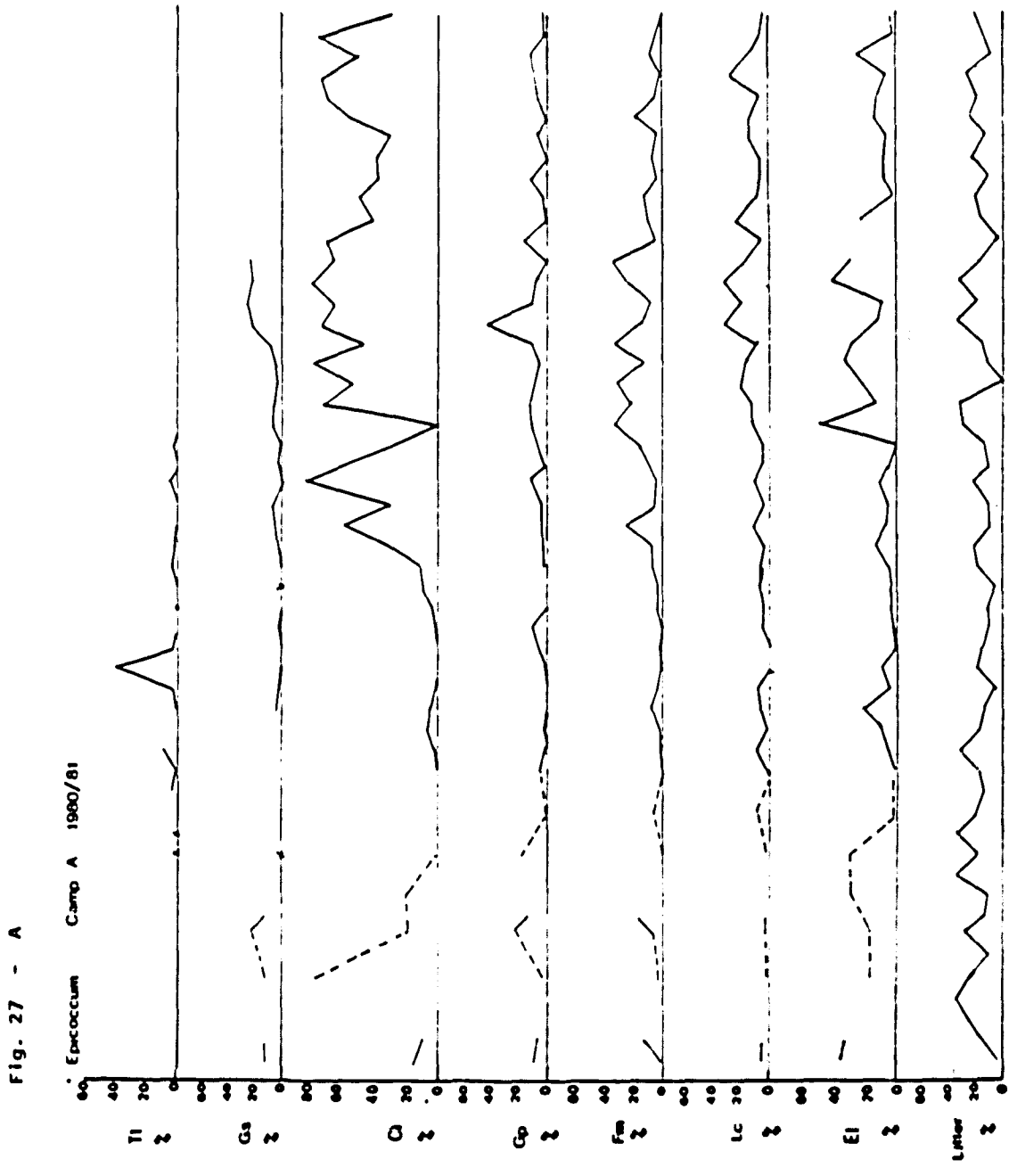


Fig. 27 - A

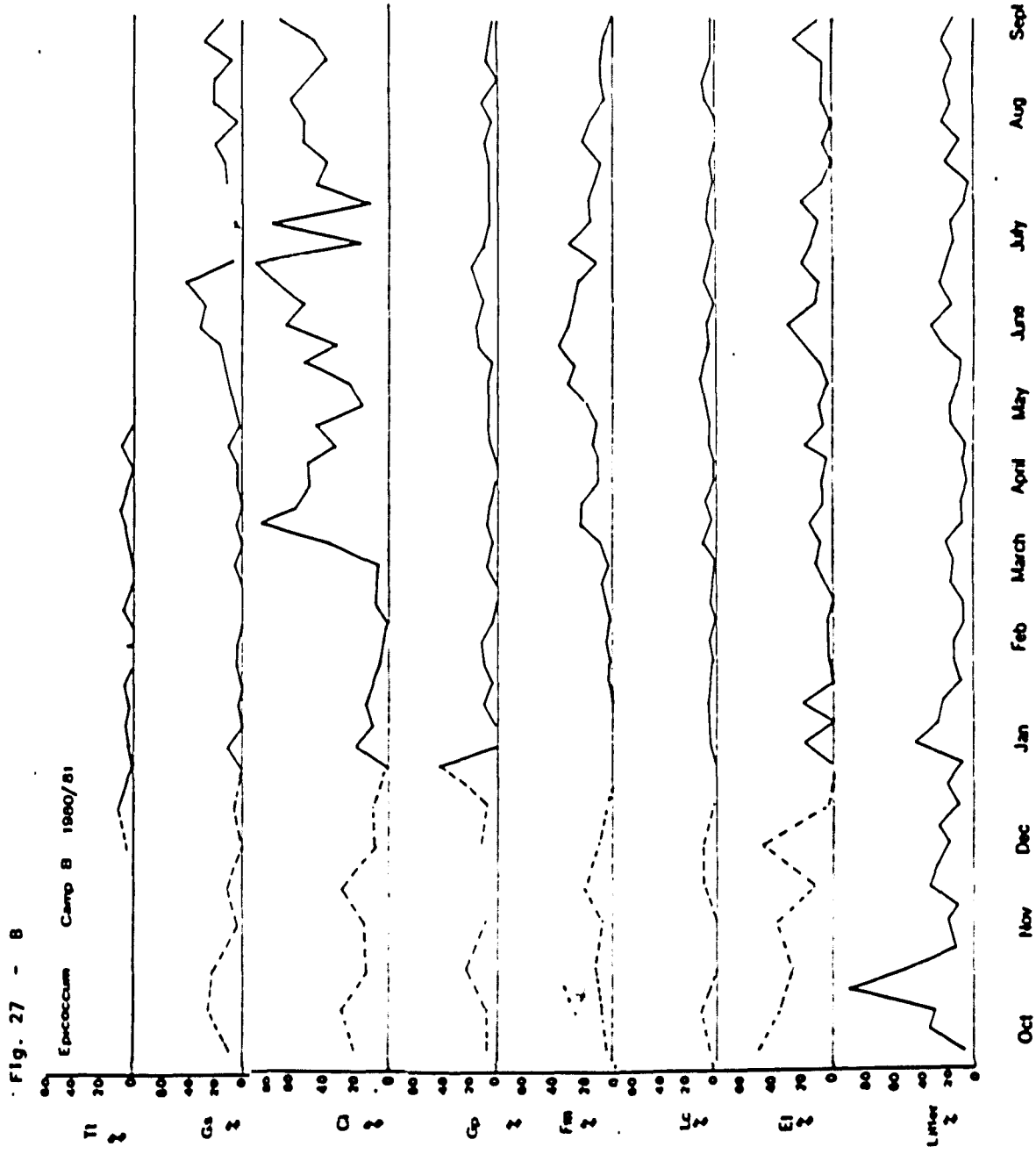


Fig. 27 - B
Epacoccum Camp B 1980/81
Tl %
Ga %
Cl %
Gp %
Fm %
Lc %
El %
Litter %
Oct Nov Dec Jan Feb March April May June July Aug Sept

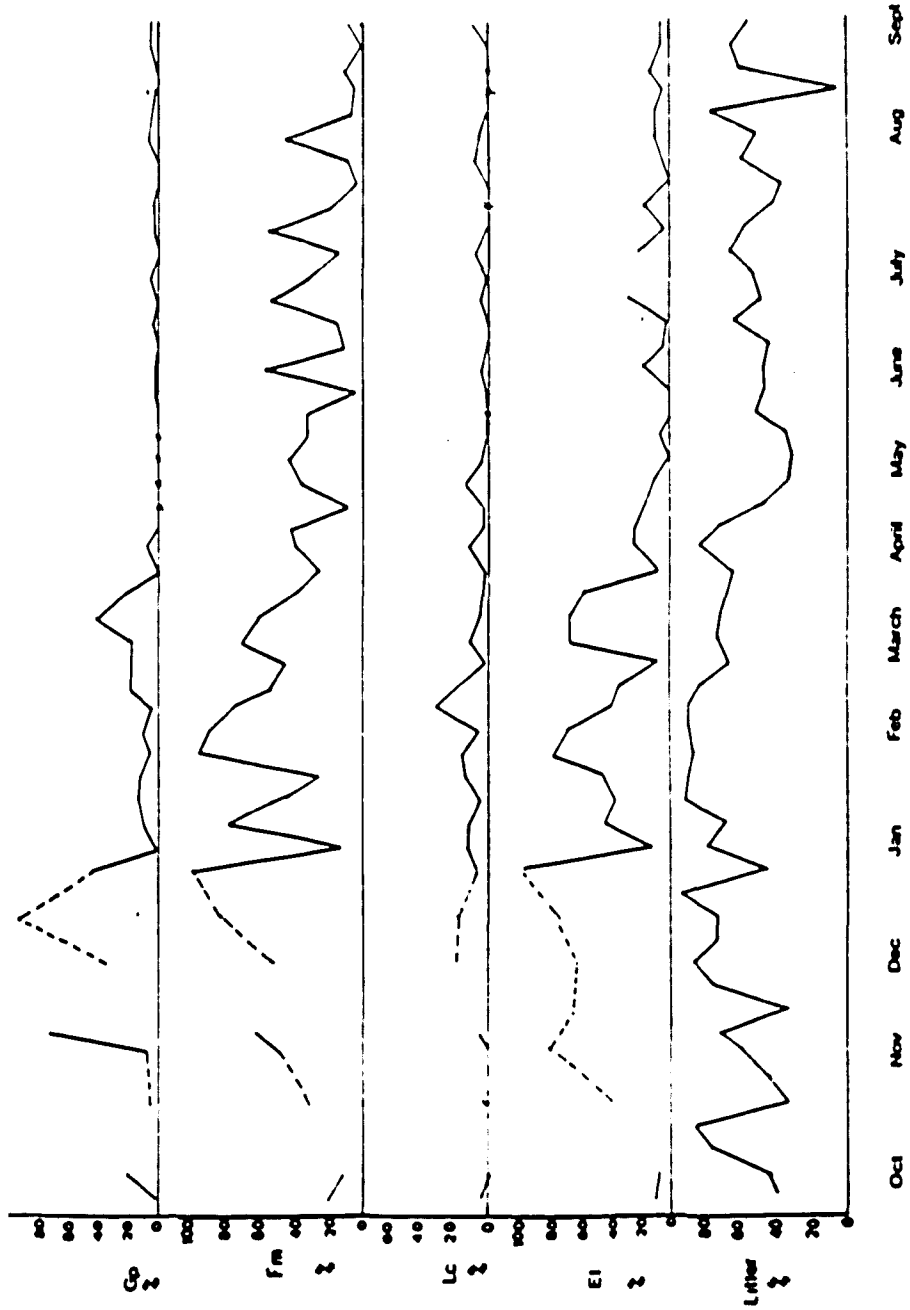

Fusarium Camp A 1980/81
Go %
Fm %
Lc %
El %
Litter %
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

Fig. 28 - A

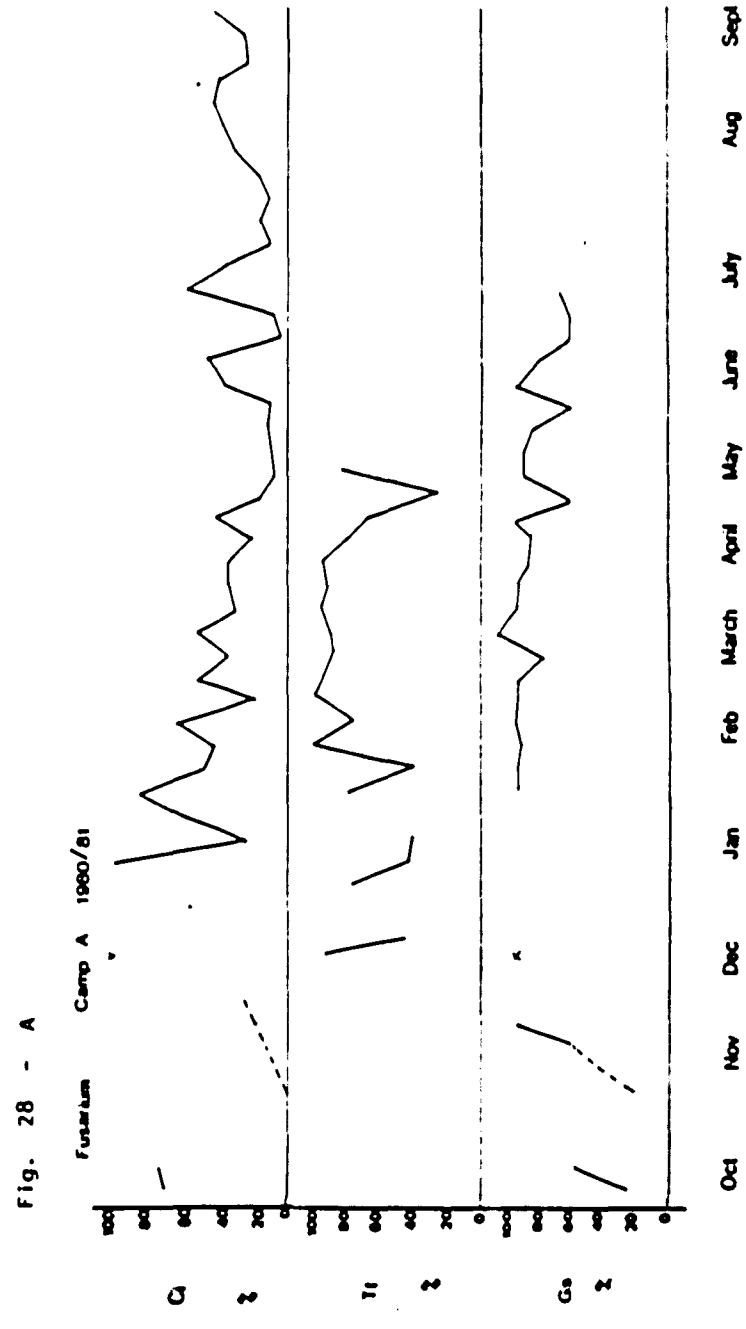

Fusarium Camp A 1980/81
Cl %
Ti %
Ga %
100
80
60
40
20
0
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

Fig. 28 - A

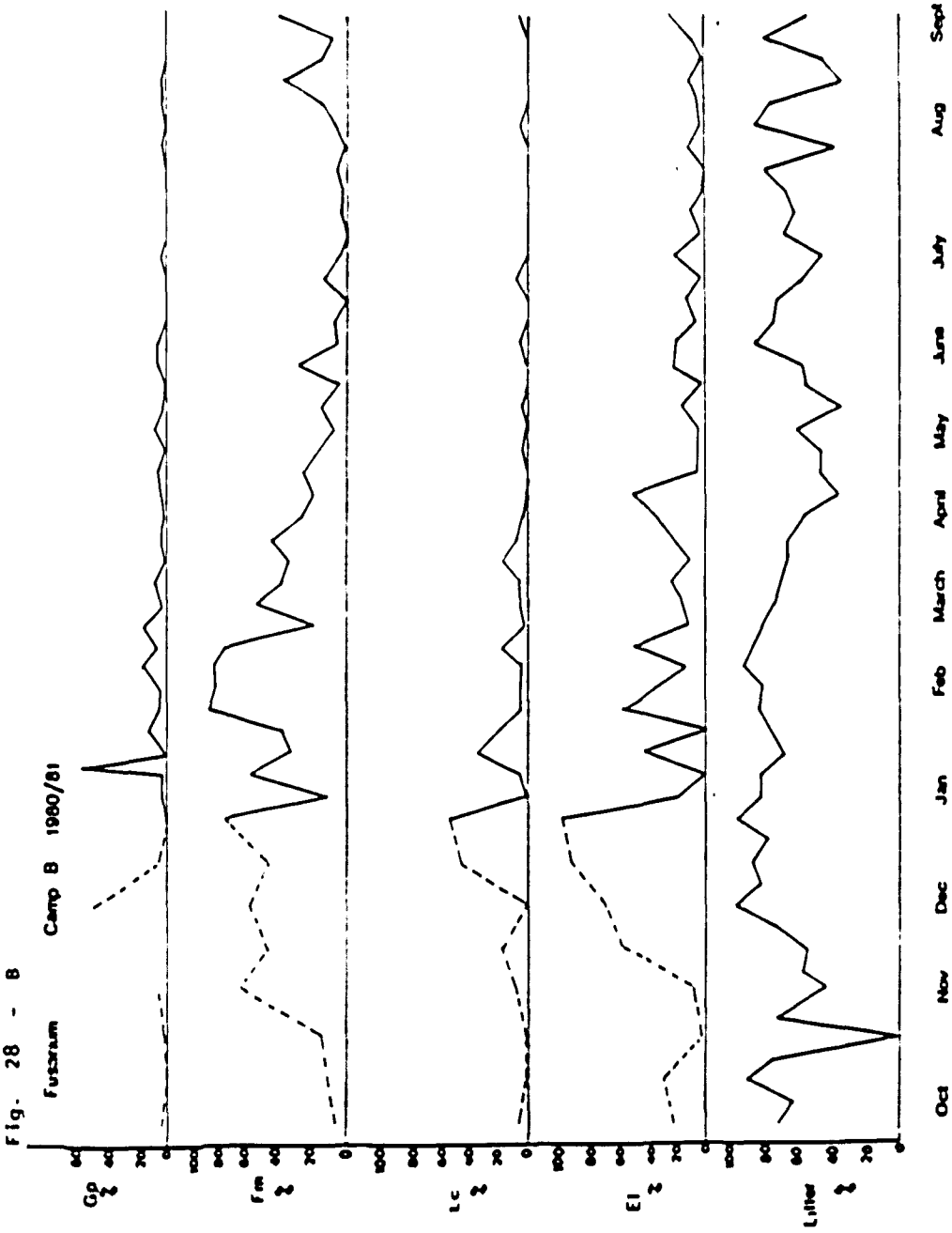


Fig. 28 - B
Fusarium Camp B 1980/81
Gp %
Fm %
Lc %
El %
Litter %
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

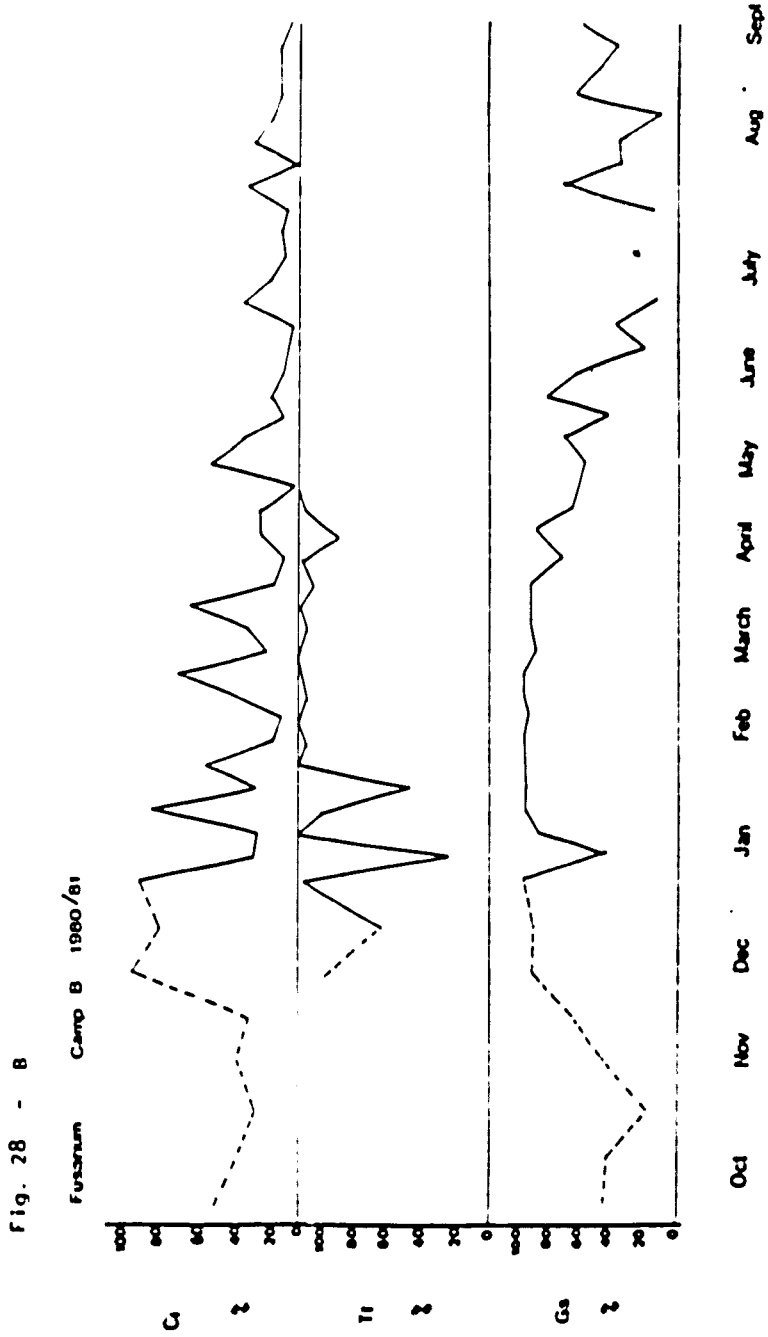


Fig. 28 - B
Fusarium Camp B 1980/81
Cs %
Tl %
Ga %
100
80
60
40
20
0
Oct Nov Dec Jan Feb March April May June July Aug Sept

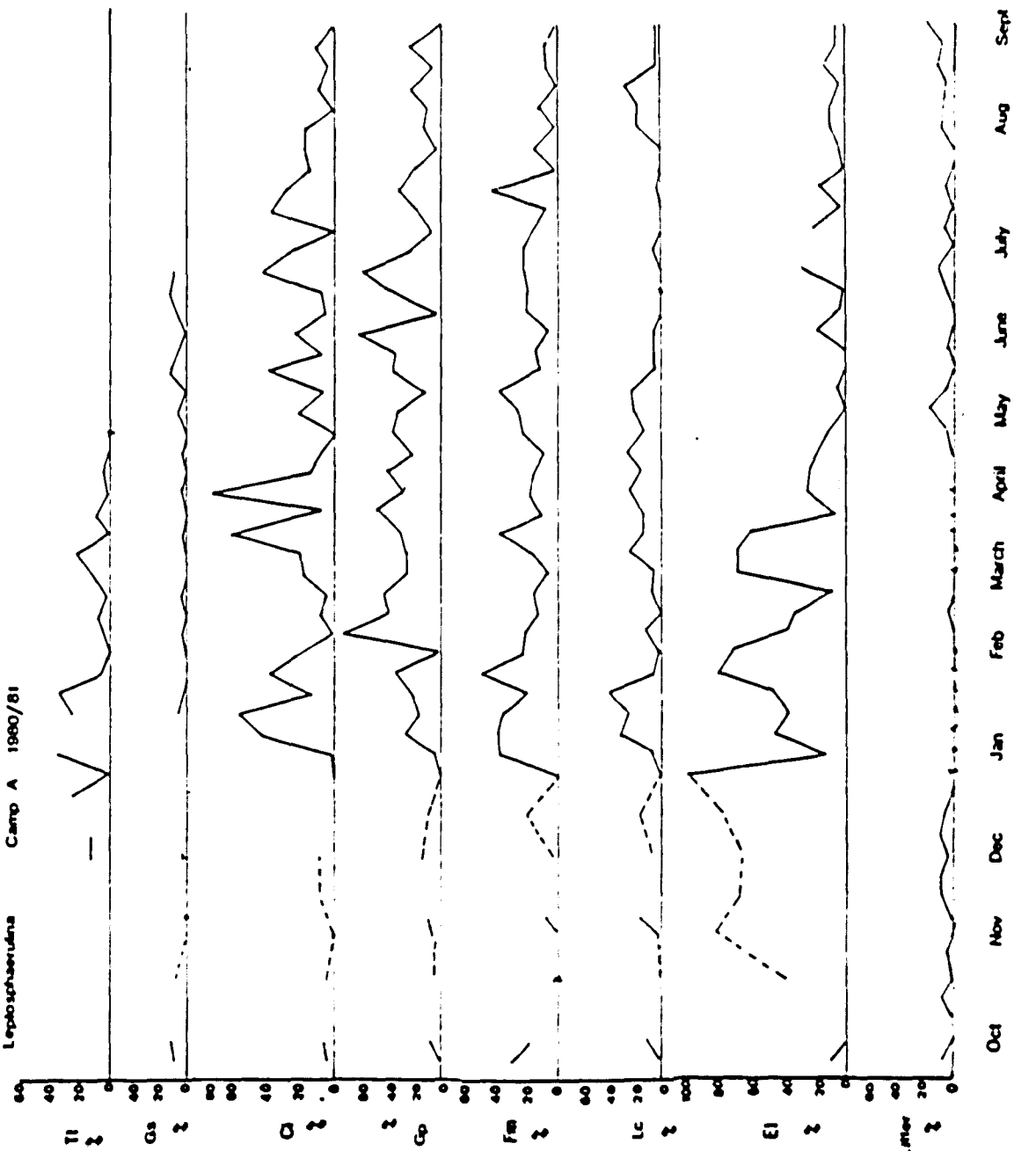

Leptosphaerulina Camp A 1980/81
Tl
Ga
Cl
Gp
Fm
Lc
El
Litter
%
Oct Nov Dec Jan Feb March April May June July Aug Sept

Fig. 29 - A

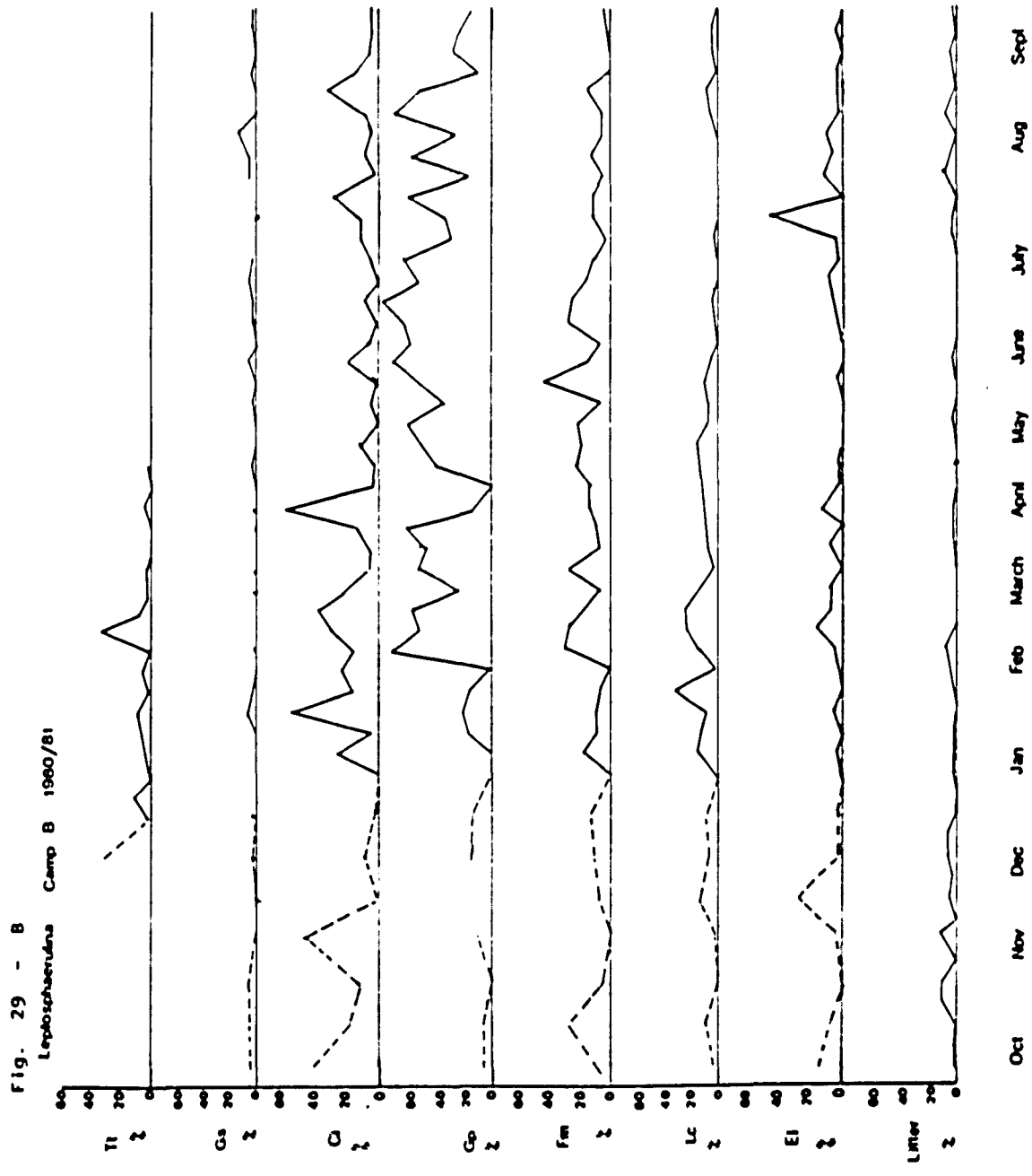


Fig. 29 - B
Leptospherulina Camp B 1980/81
Tt
Gs
Cl
Gp
Fm
Lc
El
Litter
%
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

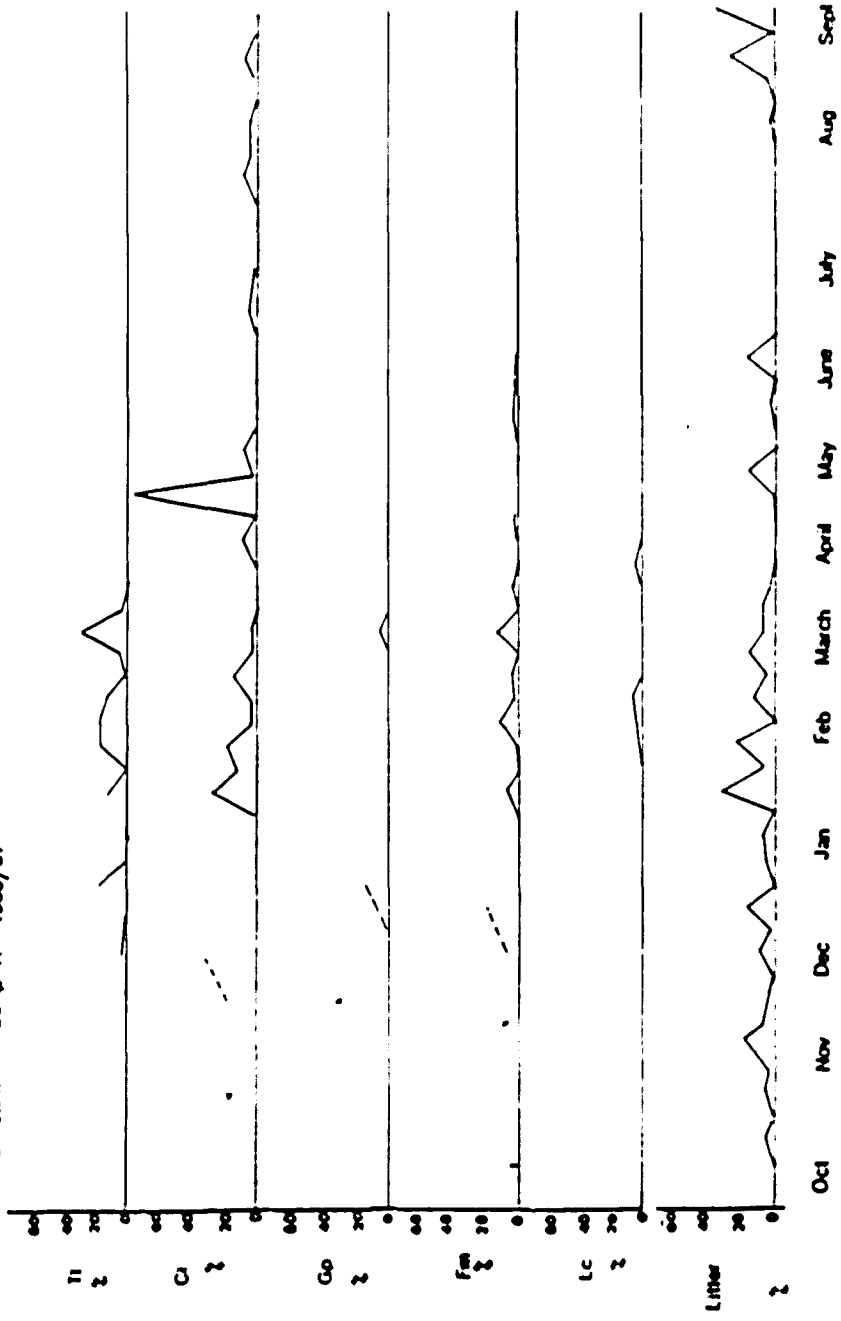


Fig. 30 - A
Metarhizium Camp A 1980/81
Il
Ci
Go
Fm
Lc
Litter
%
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

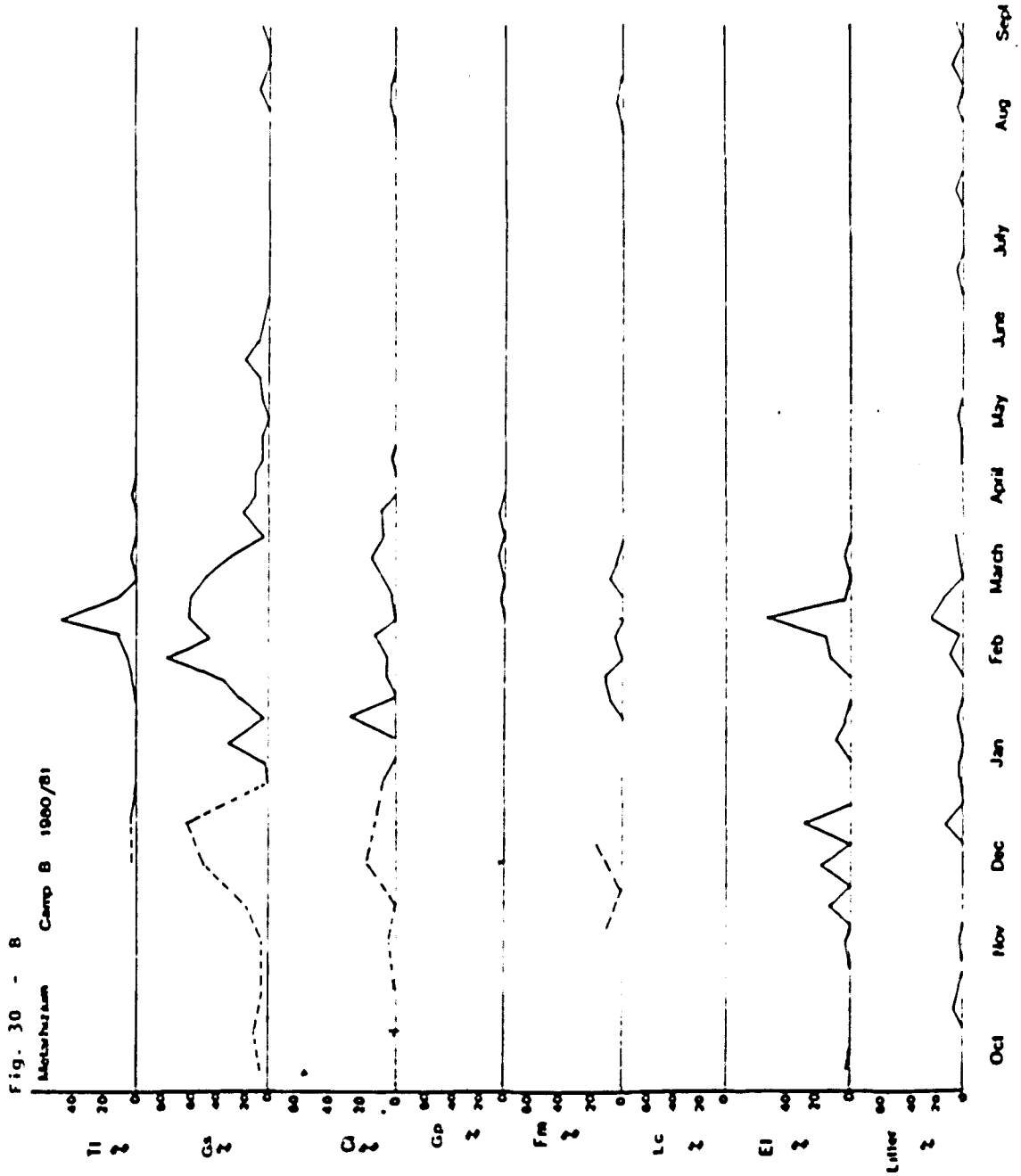


Fig. 30 - B
Meteorstasm Camp B 1980/81
Tl
Ga
Cl
Gp
Fm
Lc
El
Litter
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

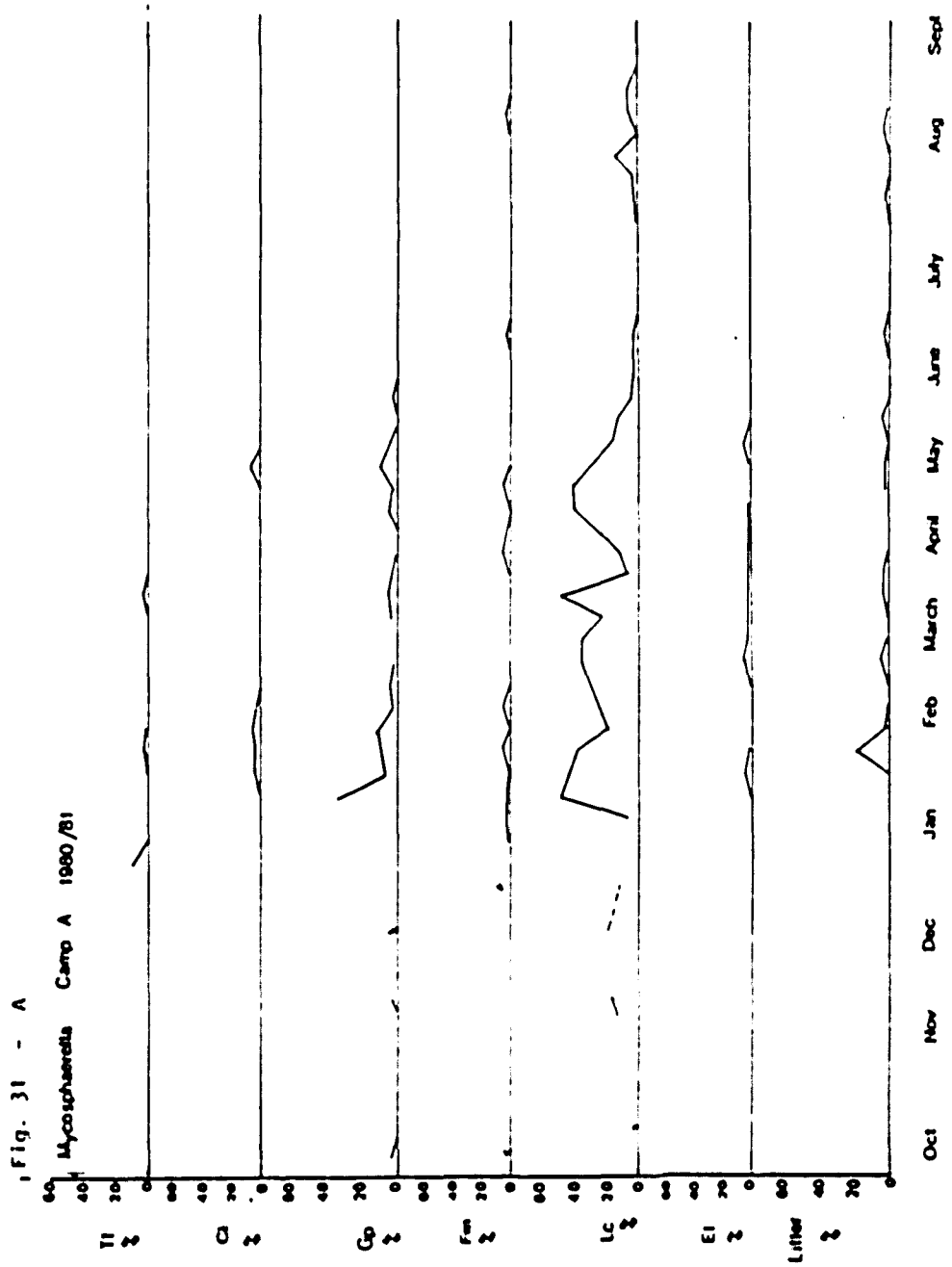


Fig. 31 - A
Mycosphaerella Camp A 1980/81
Tl %
Cl %
Gp %
Fm %
Lc %
El %
Litter %
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

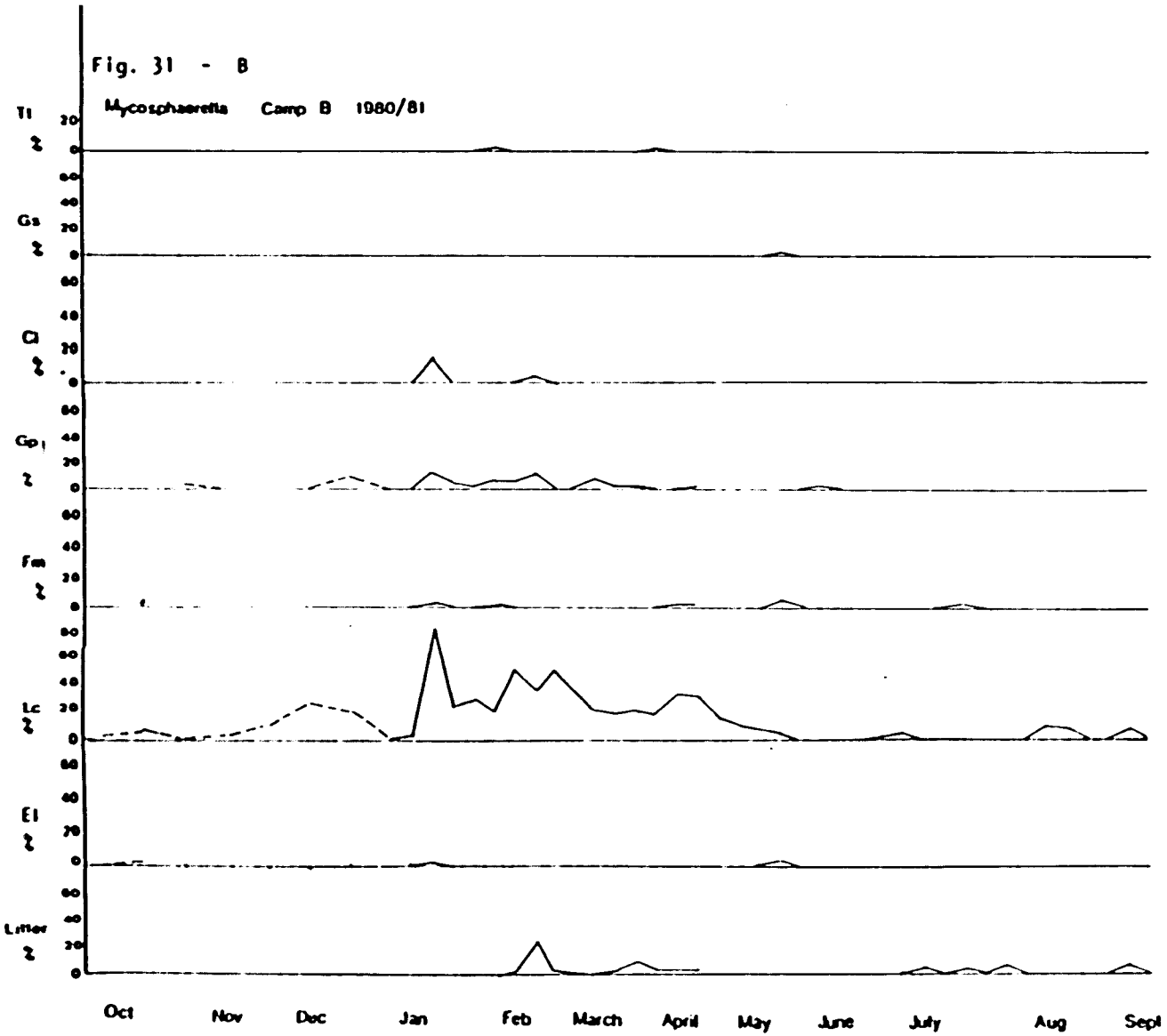


Fig. 31 - B
Mycosphaerella Camp B 1980/81
Tl
%
Gs
%
Cl
%
Gp1
%
Fm
%
Lc
%
El
%
Litter
%
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

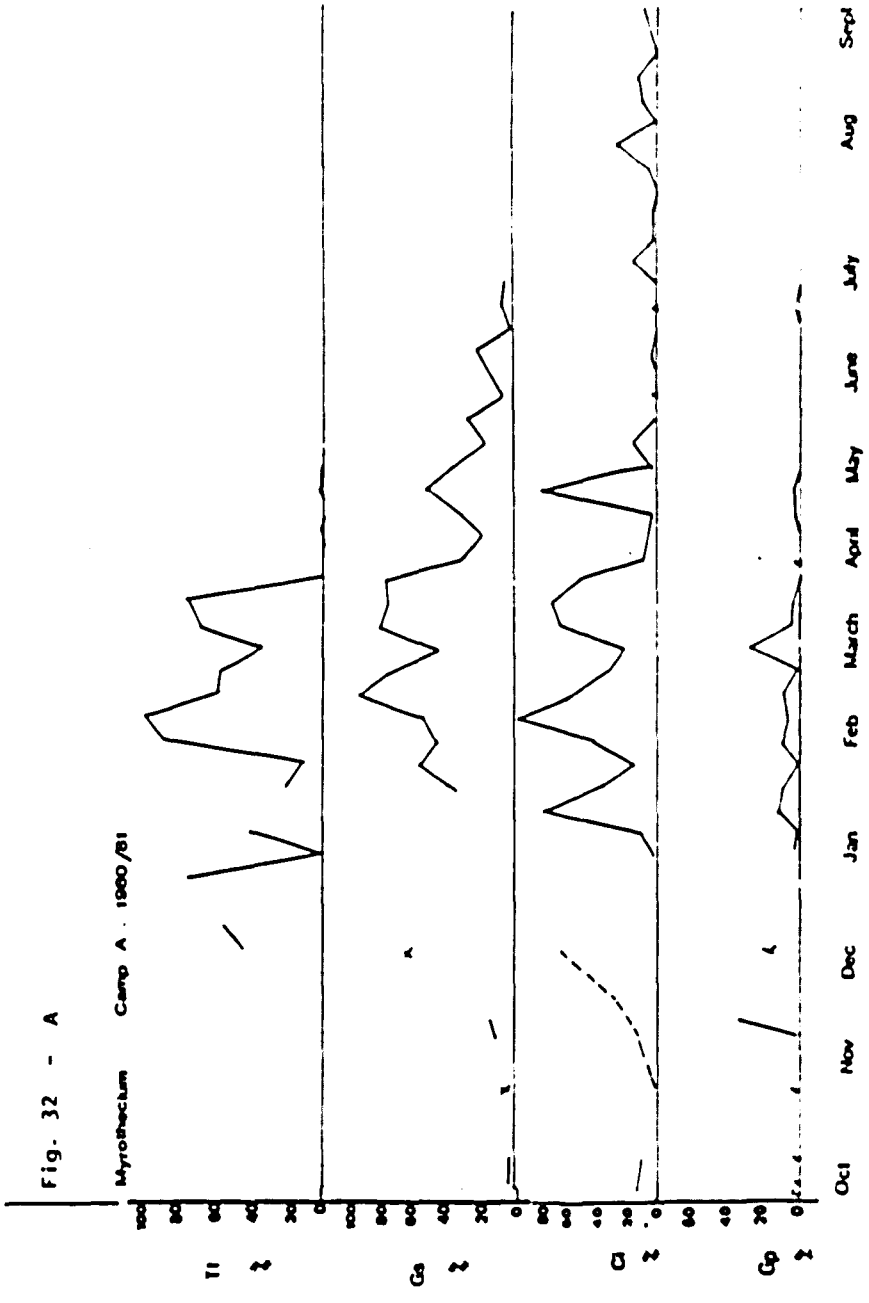
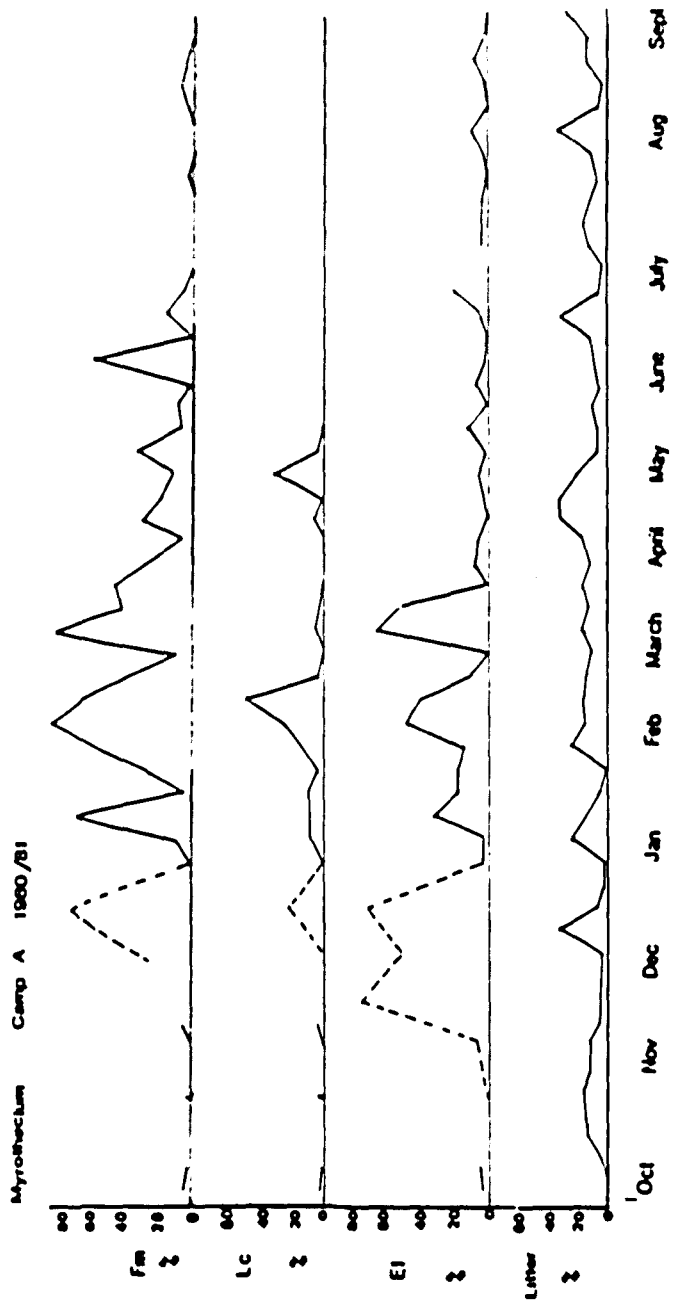


Fig. 32 - A
Myrothecium Camp A. 1980/81
Tl %
Ga %
Cl %
Gp %
100
80
60
40
20
0
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

Fig. 32 - A


Myrothecium Camp A 1980/81
Fm
Lc
El
Litter
%
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

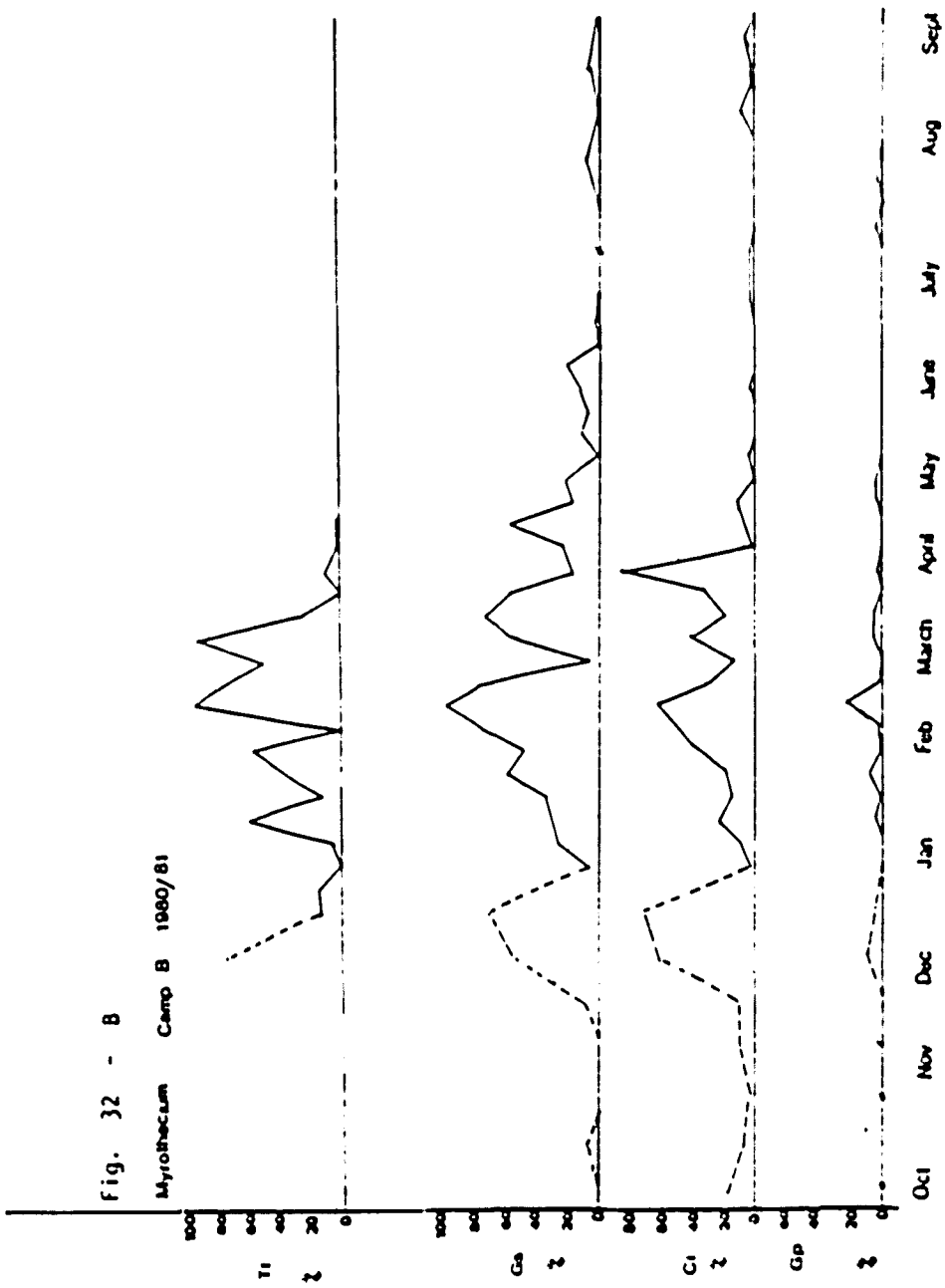


Fig. 32 - B
Myrothecium Camp B 1980/81
Ti
Ga
Cl
Gp
%
100
80
60
40
20
0
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

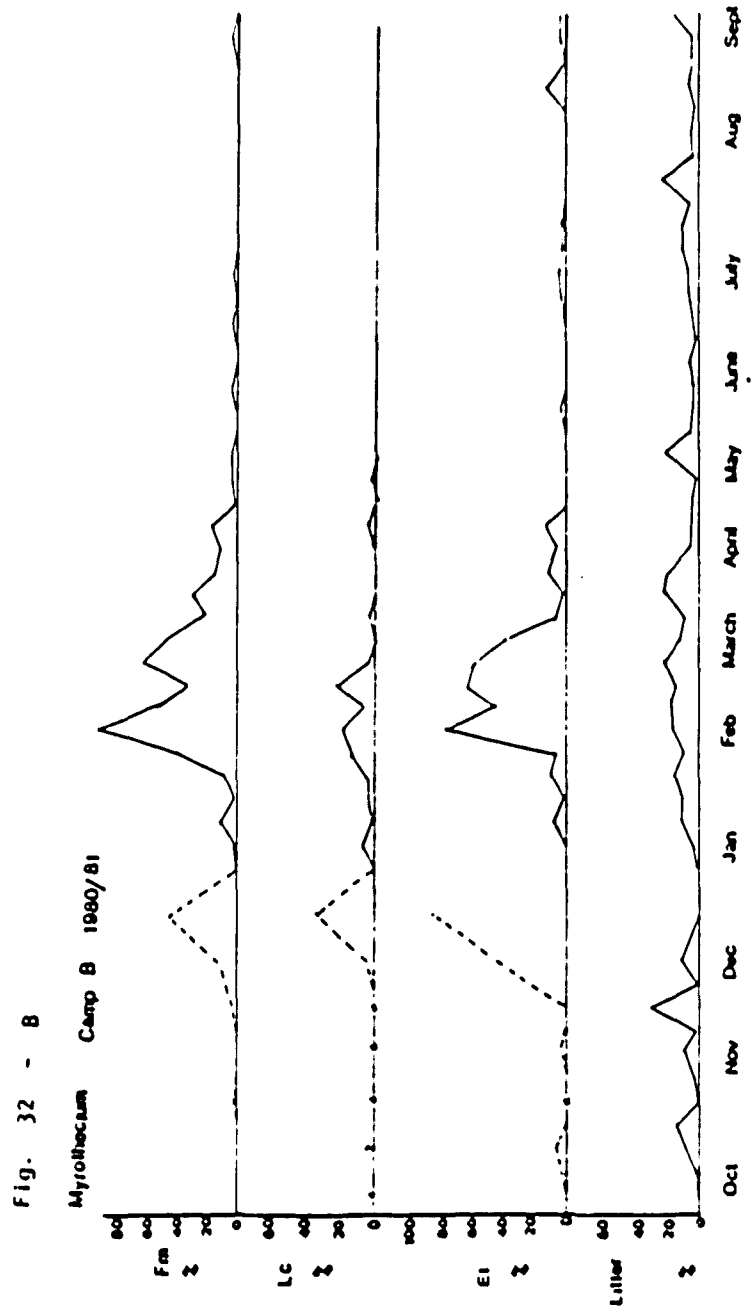


Fig. 32 - B
Myrothecium Camp B 1980/81
Fm
%
Lc
%
Ei
%
Litter
%
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

Fig. 33 - A

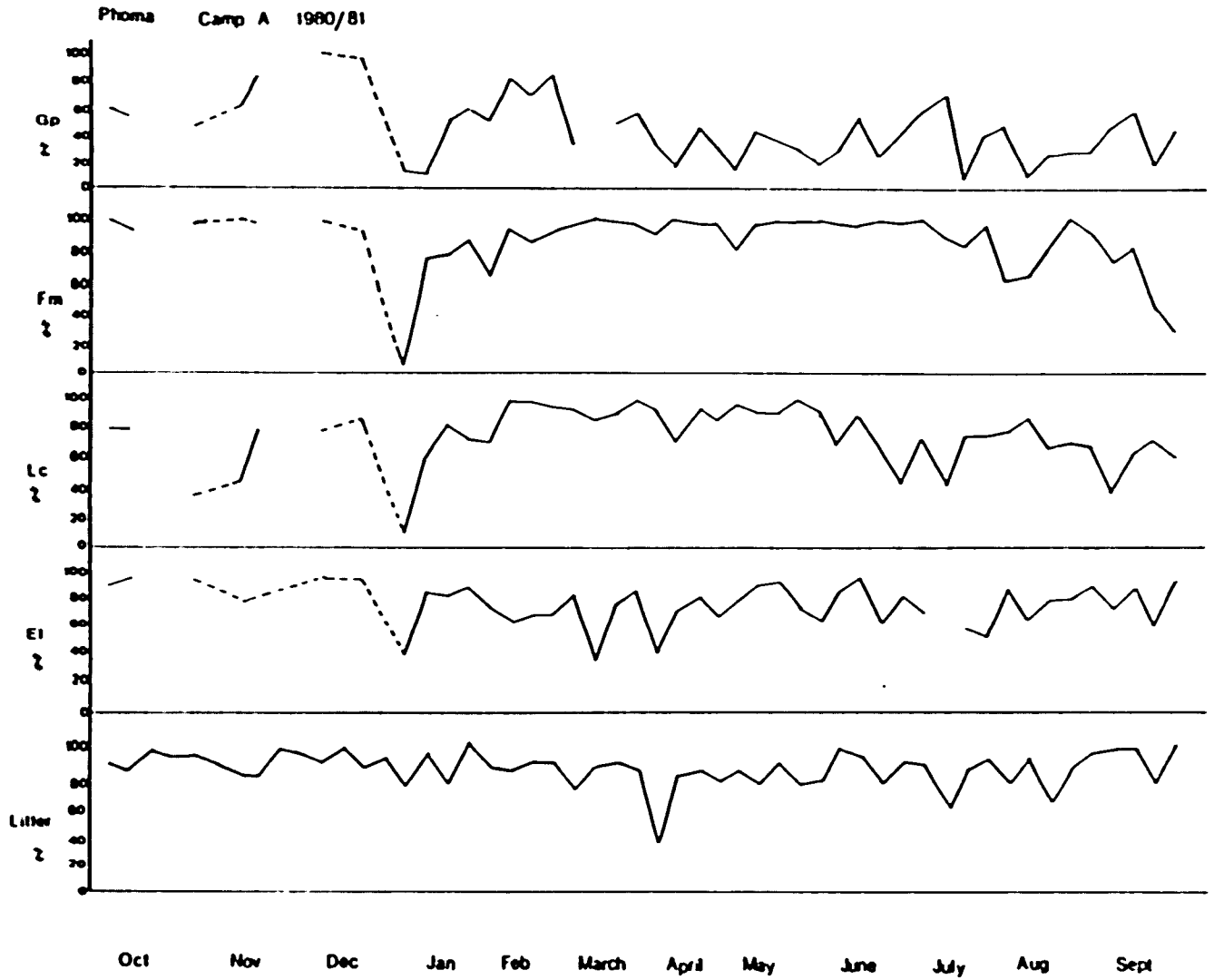
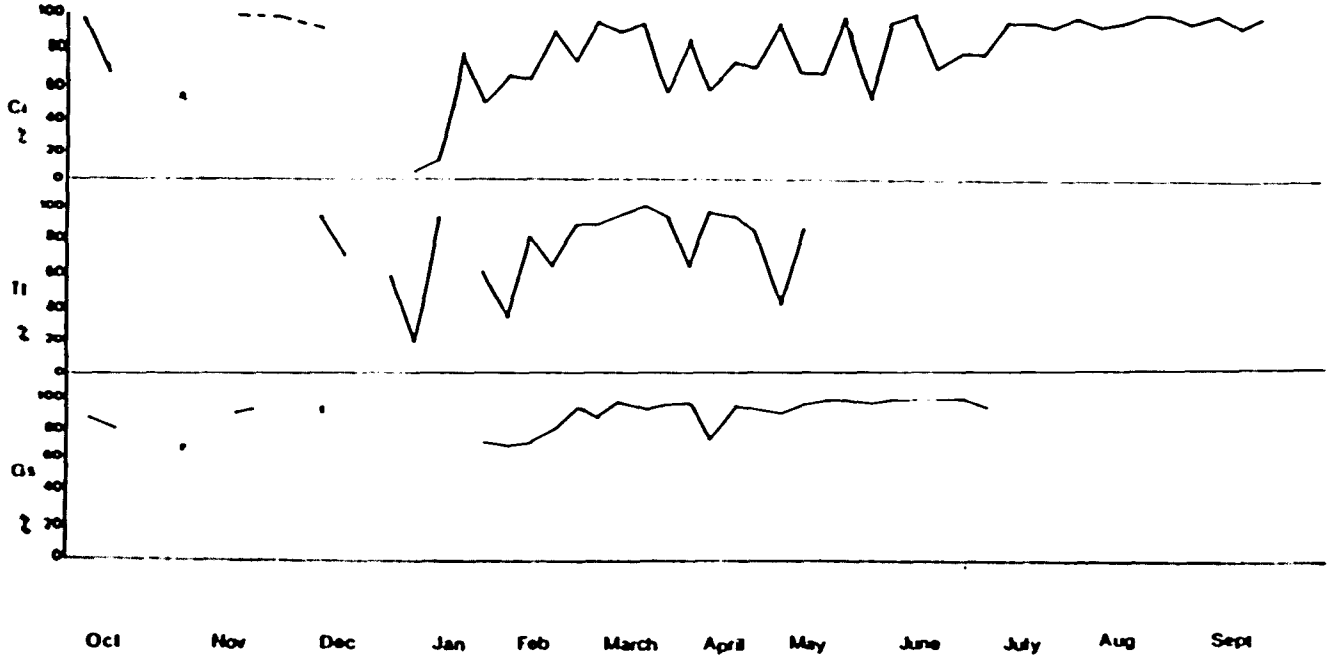

Phoma Camp A 1980/81
Gp %
Fm %
Lc %
El %
Litter %
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

Fig. 33 - A

Phoma Camp A 1980/81



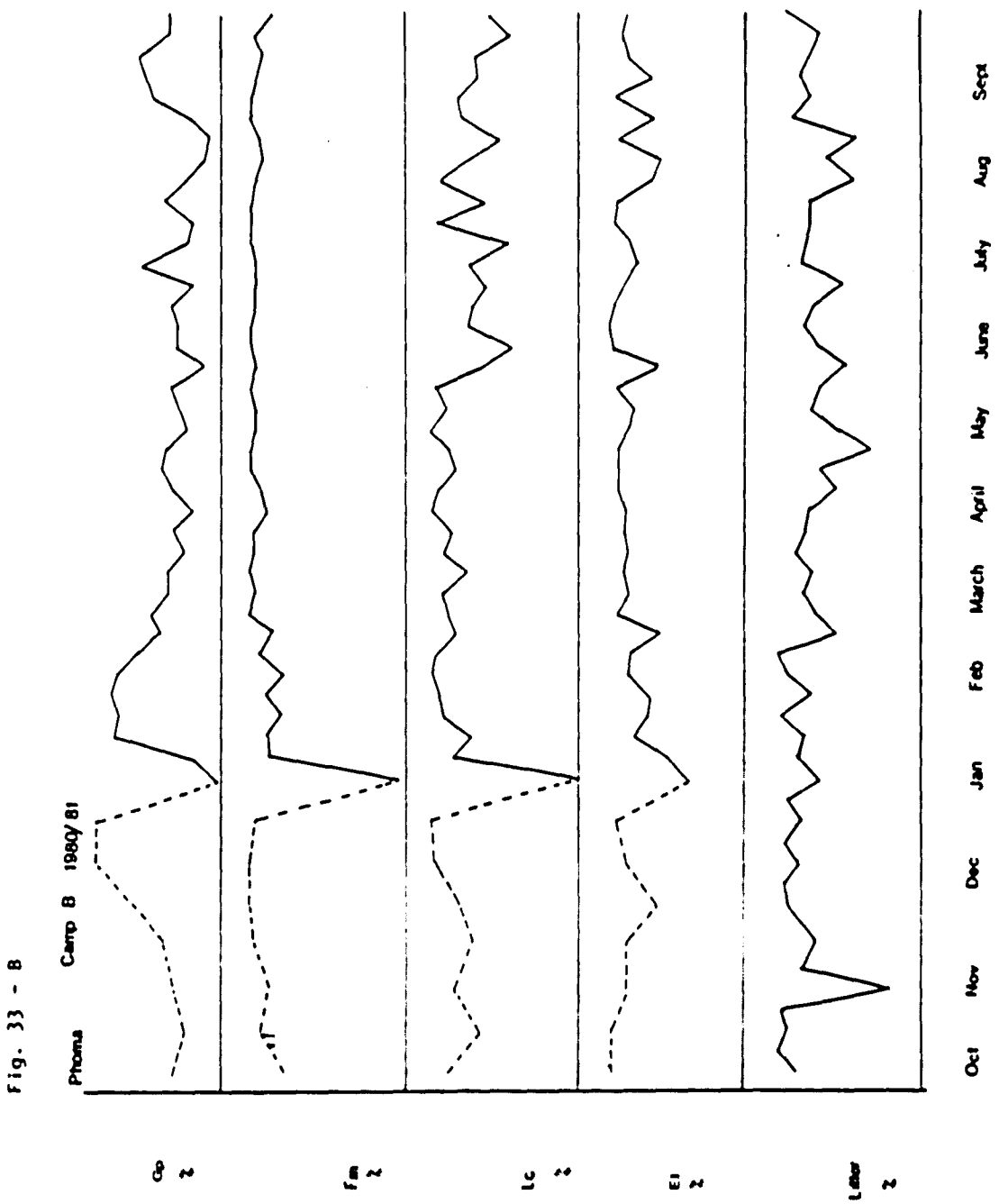


Fig. 33 - 8
Phoma
Camp B 1980/81
Cp %
fm %
Lc %
El %
Lalter %
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

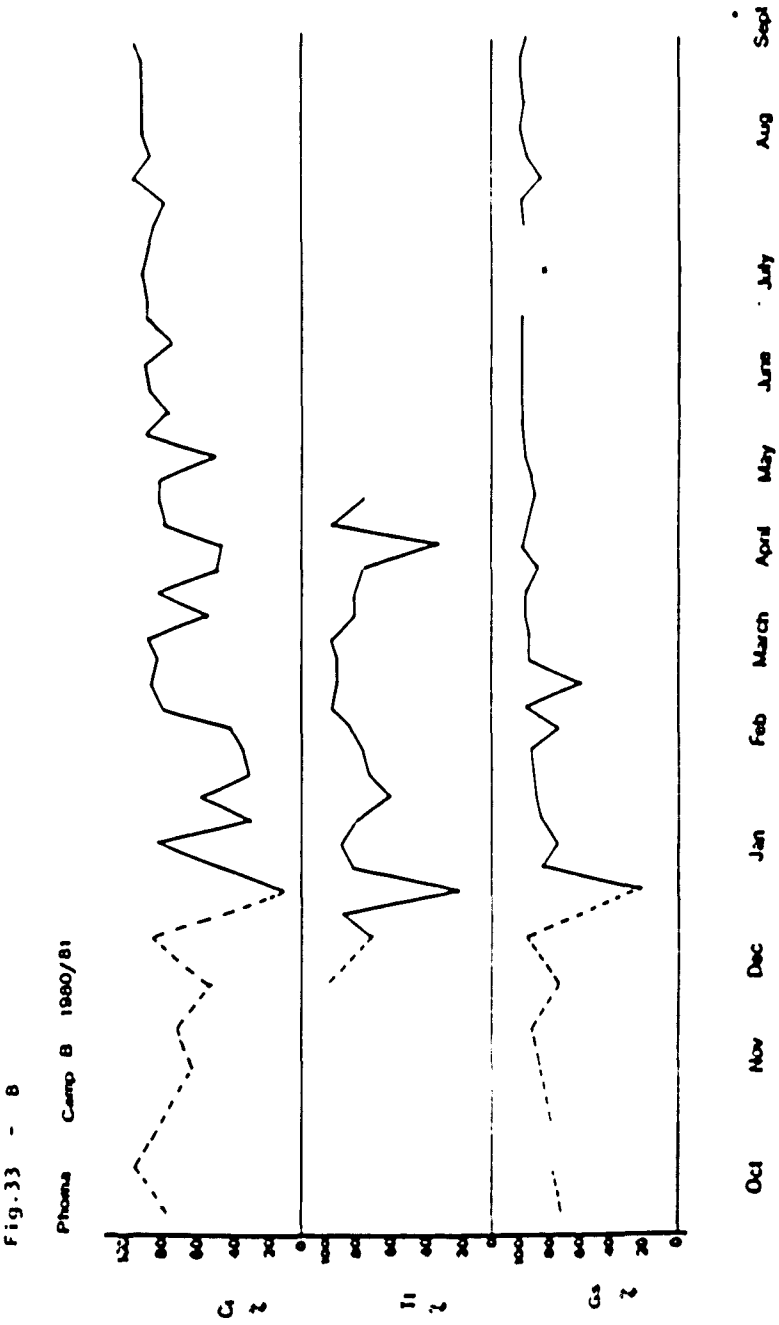


Fig.33 - B

Phona Camp B 1980/81

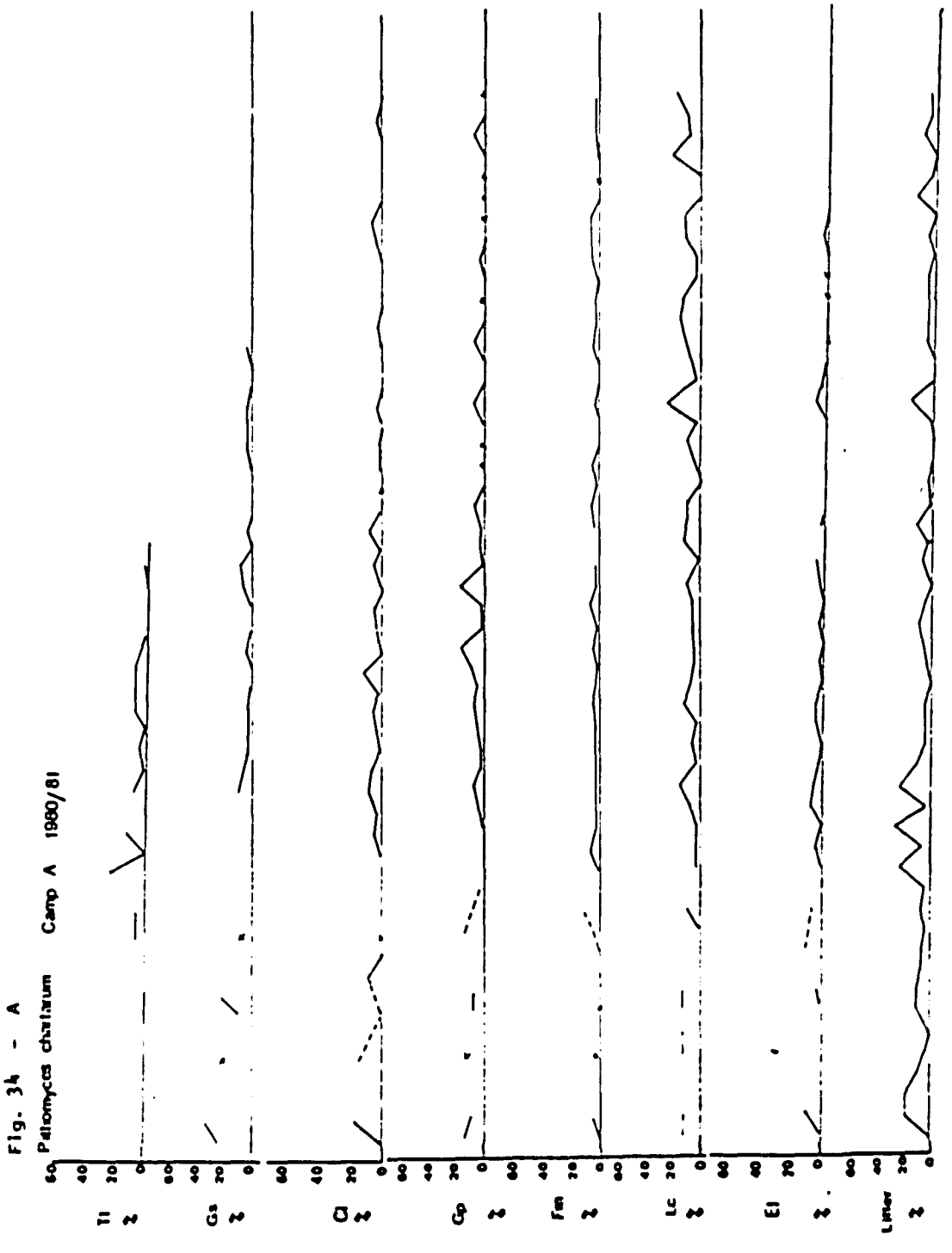


Fig. 34 - A
Palamycas chalcum Camp A 1980/81
Tl
Gs
Cl
Gp
Fm
Lc
El
Litter
Oct Nov Dec Jan Feb March April May June July Aug Sept

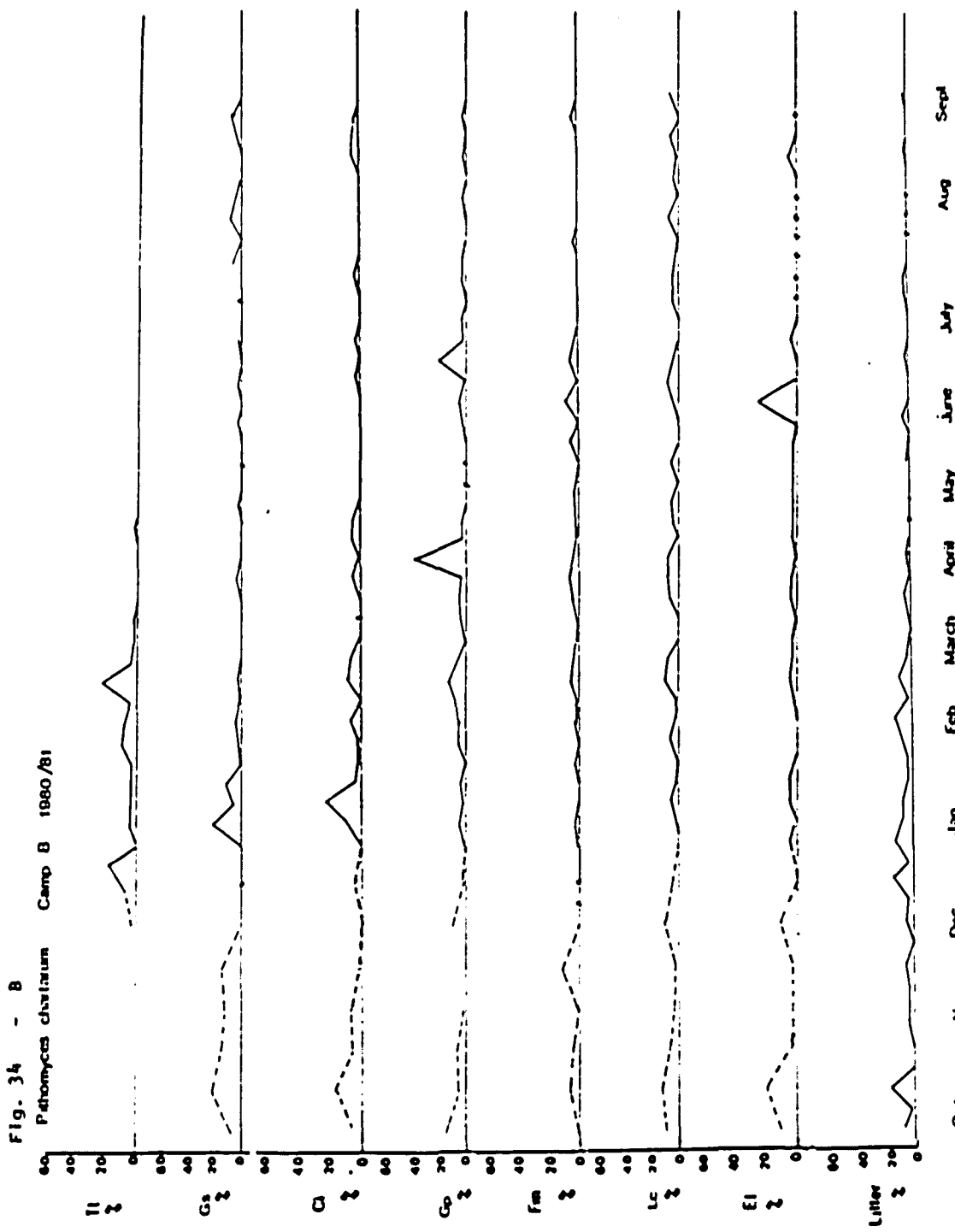


Fig. 34 - B
Pithomyces chartarum Camp B 1980/81
Il
Ga
Cl
Cp
Fm
Lc
El
Litter
%
Oct Nov Dec Jan Feb March April May June July Aug Sept

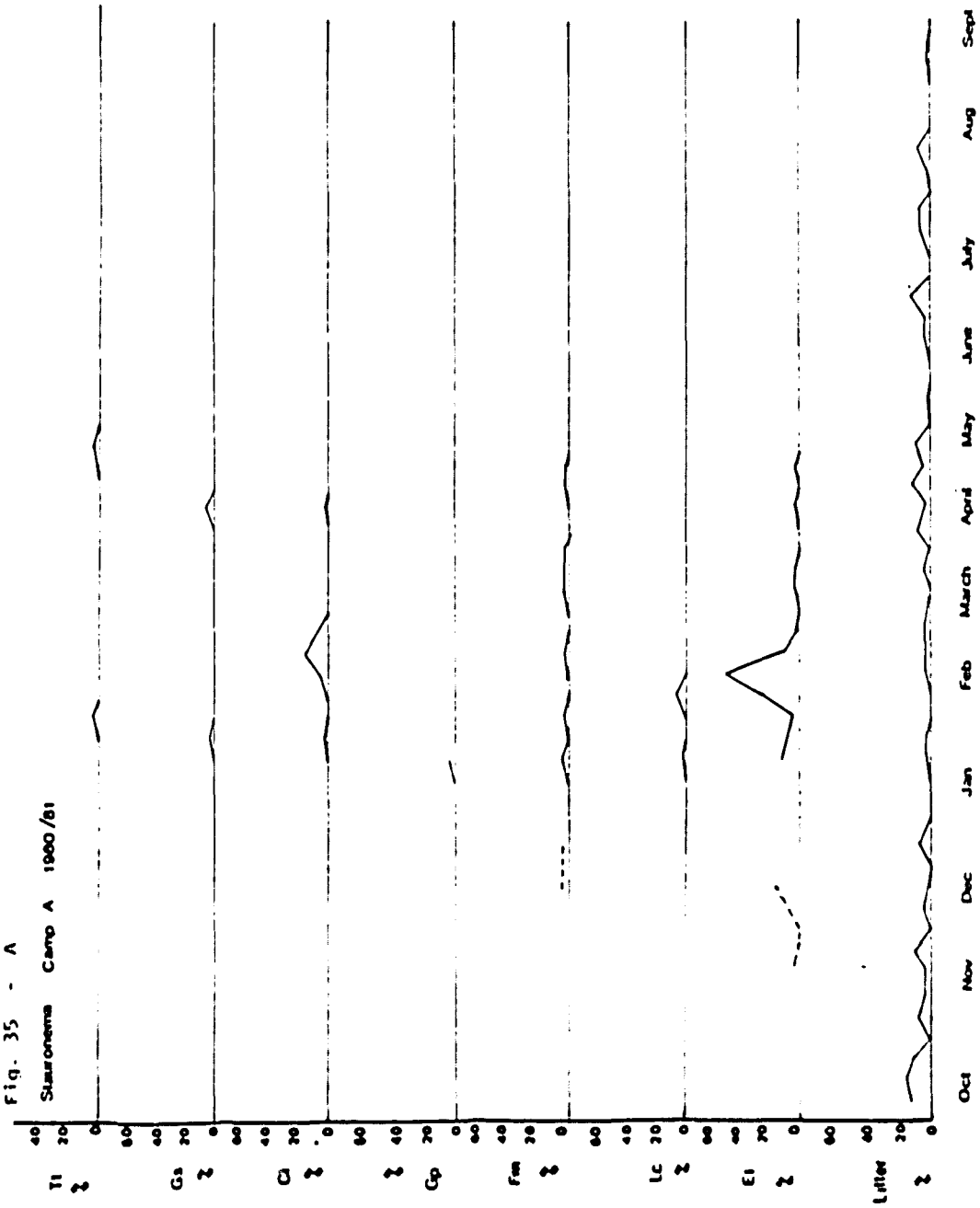


Fig. 35 - A
Sisaronema Camp A 1980/81
Tl
Gs
Cl
Gp
Fm
Lc
Ei
Litter
%
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

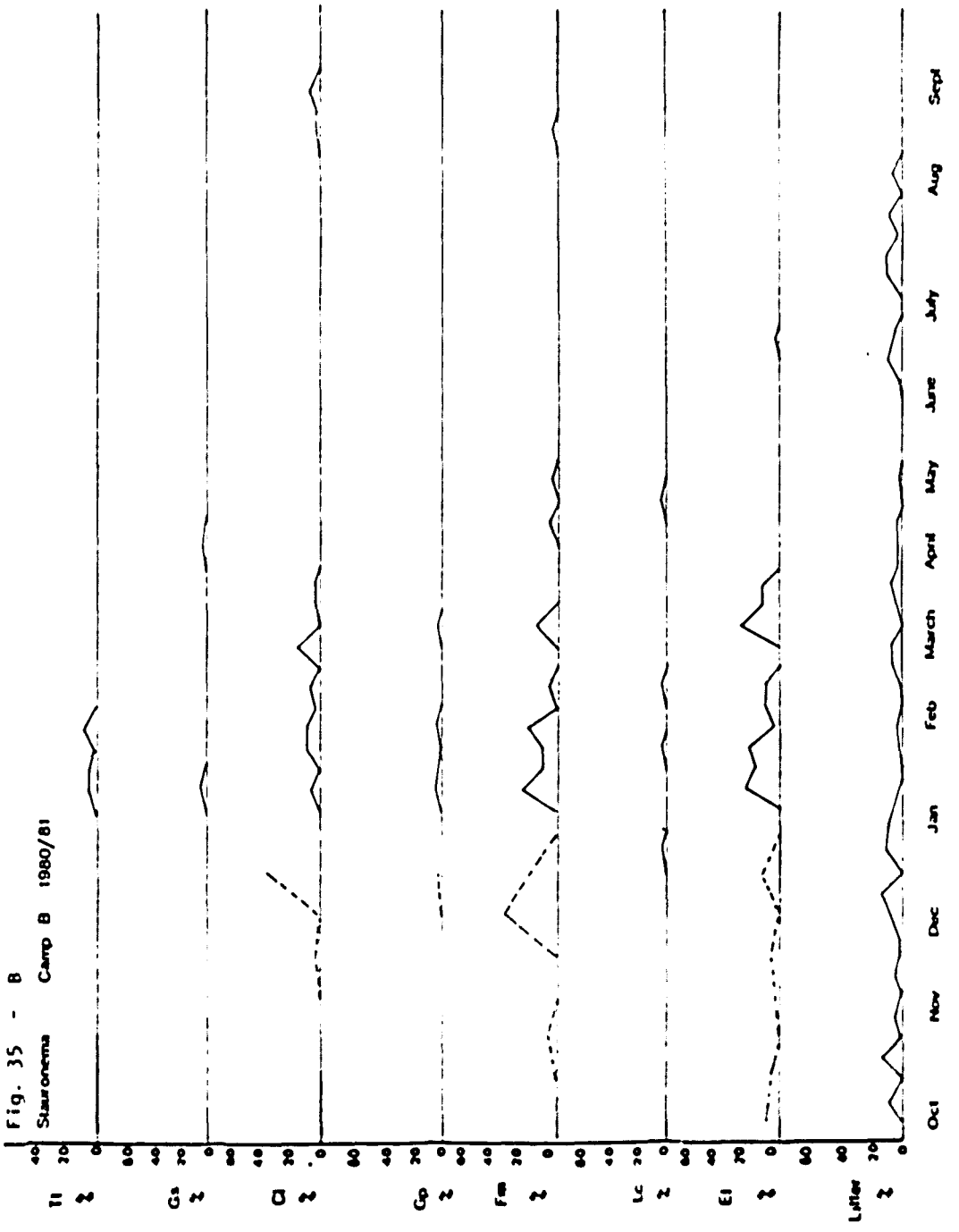


Fig. 35 - B
Staurocnma Camp B 1980/81
Tl
Ga
Cl
Cp
Fm
Lc
El
Luller
%
0
20
40
60
80
Oct
Nov
Dec
Jan
Feb
March
April
May
June
July
Aug
Sept

TABLE 3

SUMMARY OF INCIDENCE OF FUNGI IN THE 1980/81 YEAR LONG SURVEY

WINTER FUNGI	
Alternaria spp.	on *Lycium* in Camp A
Aureobasidium spp.	high on all substrates except *Tribulus* and litter
Camarosporium spp.	high on *Galenia procumbens* in Camp A
Cladosporium spp.	high on all substrates in Camp B
Epicoccum purpurascens	on *Cynodon* in Camps A & B
Leptosphaerulina spp.	highest on *G. procumbens* in Camp B
Rhizoctonia spp.	high on litter from April onwards in Camp B
AUTUMN FUNGI	
Fusarium spp.	high on *F. muricata* and lower plants (Fig. 1) Camp B
Metarhizium anisopliae	on all except *Tribulus*, *Cynodon* and *Felicia* Camp A disappeared after autumn in Camp B
Myrothecium spp.	peak in late summer in Camps A & B; low on litter, peak on *Tribulus* in late summer Camp A
Leptosphaerulina 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
SUMMER FUNGI	
Camarosporium spp.	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
NO OBVIOUS PATTERN	
Alternaria 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 WHICH WERE 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 4

A SUMMARY OF THE SAMPLING DATES AND NUMBERS OF SAMPLES COLLECTED

		January	February	March	April	May	June	July	August	September	October	November	December	Number of sampling units per year	
1978	3 points								**********	**********				15 700	78/79
1979		**********											**		
1980	2 camps	********									********			4 050	79/80
1981		**********	**********	**********	**********	**********	**********	**********						28 600	80/81
1982				***										discontinued +	
		SUMMER		/		WINTER		/			SUMMER				

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

TABLE 5

A SUMMARY OF THE GENERA IDENTIFIED

TAXA	NUMBER OF GENERA	% OF THE 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 werenot included in the calculation because identification to genus level was not possible.

TABLE 6

COMPLETE LIST OF FUNGAL TAXA IDENTIFIED

HYPHOMYCETES

* New Genera for South Africa
** New Species for South Africa

Acremoniella atra (Corda) Sacc.
A. verrucosa Tognini
Acremonium Link spp.
Alternaria alternata (Fr.) Keissler
A. zinniae M.B.Ellis
Arthrobotrys superba Corda
Arthrinium saccharicola Stevenson
Aspergillus candidus Link
A. flavus Link
A. nidulans Eidam
A. niger van Tieghem
A. terreus Thom
Aspergillus Link spp.
Aureobasidium pullulans (de Bary) Arnaud
Aureobasidium Viala et Boyer

Beauveria bassiana (Bals.) Vuill.
Botrytis state of *Sclerotinia fuckeliana* (de Bary) Fuckel
Botrytis Michell ex Pers.

Cephalosporium Corda *sp.*
Cercospora Fres. *sp.*
Cerebella andropogonis Ces.
Chrysonilia sitophila (Mont.) v. Arx
Cladorhinum foecundissimum Sacc. et Marchal
Cladosporium cladosporioides (Fresen.) De Vries
C. herbarum (Pers.) Link
Cladosporium Link spp.
Conidial state of *Monascus* v. Tieghem
Curvularia lunata (Wakker) Boedjin
** *C. tuberculata* Jain

Dactylella Grove sp.
** *Dichotomophthora portulacae* Mehrlich & Fitzpatrick ex M.B.Ellis
Doratomyces stemonitis (Pers. ex Fr.) Morton & Smith
Drechslera state of *Cochliobolus carbonus* Nelson
Drechslera state of *Cochliobolus cynodontis* Nelson
D. halodes (Drech.) Subram. & Jain
D. hawaiiensis (Bugnicourt) Subram. & Jain ex M.B. Ellis

D. papendorfii (Van der Aa) M.B.Ellis
D. phlei (Graham) Shoemaker
D. rostrata (Drech.) Richardson & Fraser

Epicoccum purpurascens Ehrenb. ex Schlecht

Fusariella cf. *obstipa* (Pollack) Hughes
Fusarium acuminatum Ell. & Ev. sensu Gordon
F. equiseti (Corda) Sacc. sensu Gordon
F. moniliforme Sheldon
F. semitectum Berk. & Rav.
* *F. stoveri* Booth
F. subglutinans (Wollenw. & Reinking) Nelson,Toussoun & Marasas

Gliocladium penicilloides Corda
G. roseum Bainier
Gonatobotrys simplex Corda
Graphium penicilloides Corda
** *Gyrothrix flagella* (Cooke & Ellis) Pirozynski

Helicomyces roseus Link
** *Helicoon sessile* Morgan
Hyalodendron lignicola (Diddens) de Hoog

** cf. *Lacellina macrospora* (Berk. & Br.) Petch.

Memnoniella echinata (Riv.) Galloway
Metarhizium anisopliae (Metschinkov) Sorokin
Monacrosporium Oudem. sp.
** *Moniliella* Stolk & Dakin sp.
Myrothecium carmichaelii Grev.
* *M. cinctum* (Corda) Sacc.
M. roridum Tode ex Fr.
M. verrucaria (Alb. & Schw.) Ditm. ex Fr.

Nigrospora state of *Khuskia oryzae* Hudson

** *Oedocephalum glomerulosum* (Bulliard) Sacc.

Paecilomyces Bainier sp.
Penicillium chrysogenum Thom
P. oxalicum Currie & Thom
Penicillium Fr. spp.
Periconia byssoides Pers. ex Mérat
P. cookei Mason & M.B. Ellis
* *Periconia* cf. *madreeya* Subram.
Pithomyces atro-olivaceus (Cooke & Hark.) M.B. Ellis
P. chartarum (Berk. & Curt.) M.B. Ellis
P. cynodontis M.B. Ellis
P. graminicola R.Y.Roy Rai
P. karoo Marasas & Schumann
* *P. maydicus* (Sacc.) M.B. Ellis
P. sacchari (Speg.) M.B. Ellis
Pyricularia oryzae Cavara

Rhinocladiella state of *Dictyotrichiella mansonii* Schol-Schwartz
* *R. collaris* (Pers. ex S.F. Gray) M.B. Ellis

Scopulariopsis brevicaulis (Sacc.) Bainier
cf. *Septofusidium elegantulum* (Pidopl.) W.Gams
* *Spegazzinia* cf. *parkeri* Sivas.
S. tessartha (Berk. & Curt.) Sacc.
* cf. *Sporidesmium* Link sp.
Stilbella Lindau sp.
Stachybotrys chartarum (Ehrenb.) Hughes
* *S. sansiveriae* Agarwal & Sharma
Stemphylium state of *Pleospora herbarum* (Pers. ex Fr.) Rabenh.
S. vesicarium (Wallr.) Simmons

** *Taeniolella scripta* (Karst.) Hughes
* *Taeniolella* Hughes sp.
Tetraploa ellisii Cooke
Torula herbarum (Pers.) Link. ex S.F. Gray
Trichoderma harzianum Rifai
Trichoderma Pers. sp.
Trichothecium roseum (Pers.) Link

Ulocladium atrum Preuss
U. chartarum (Preuss.) Simmons
* *U. tuberculatum* Simmons

Volutella collatotrichoides Chilton

** *Volutina concentrica* Penz. et Sacc.
* *Volutina* Penzig. & Sacc.

ALBINO FUNGI

Alternaria alternata (Fr.) Keissler
Cladosporium cladosporioides (Fresen.) de Vries
Stachybotrys chartarum (Ehrenb.) Hughes

COELOMYCETES

* New Genera for South Africa
** New Species for South Africa

** *Amerosporium concinnum* Petrak
Ascochyta Lib. spp.
** *Ascochytulina* Petrak sp.

** *Bartalinia robillardoides* Tassi

** *Camarosporium quaternatum* (Hazsl.) Schulz.
Camarosporium Schulz. spp.
** *Chaetodiplodia* P. Karsten spp.
** *Chaetosperma chaetosporum* (Pat.) Smith & Ramsbottom
Collatotrichum coccodes (Wallr.) Hughes
C. dematium (Pers. & Fr.) Grove
C. gloeosporioides (Perz.) Sacc.
C. graminicola (Ces.) Wilson
Coniothyrium fuckelii Corda
Coniothyrium Corda spp.

** *Dinemasporium strigosum* (Pers. ex Fr.) Sacc.
Dinemasporium Lév. spp.
Diplodia Fr. spp.

** *Eriospora leucostoma* Berk. & Br.

Gelatinosporium Peck sp.

Hendersonia Sacc. spp.

** *Idiocercus macarangae* (T.S. Ramakr.) Sutton

** *Jahniella* Petrak spp.

Libertella Desm. sp.

Macrophomina phaseolina (Tassi) Gold.
Melanconium Link sp.
Melanophoma karoo Papen. & Du Toit
* *Melanophoma* Papen. & Du Toit sp.
Microsphaeropsis Höhnel sp.

** *Neottiosporina masonii* Sutton apud Sutton & Marasas

Pestalotiopsis guepinii (Desm.) Stey.
Pestalotiopsis Stey. sp.
** cf. *Phacidiella* P. Karsten sp.
** *Phaeoseptoria* Speg. sp.
* *Phoma epicoccina* Punit., Tull. & Leach
P. glomerata (Corda) Wollenw. & Hochapf.
P. sorghina (Sacc.) Boerema, Dorenbosch & Van Kesteren
Phoma Sacc. spp.
Phomopsis (Sacc.) Bubák
** *Pleurothyrium longissimum* (Lib.) Bub.
** cf. *Pleurothyrium* Bubák sp.
** *Polynema* Lév. sp.
** *Polystigmina rubrum* (Desm.) Sacc.
** *Pseudoseptoria* Speg. sp.
** cf. *Pycnofusarium* Punith. sp.
** *Pyrenochaeta* de Not. sp.

** *Sarcinulella* Sutton & Alcorn sp.
** *Seimatosporium* Corda sp.
Septoria Sacc. sp.
** *Septoriella junci* (Desm.) Sutton
* *Septoriella* Oudem. sp.
Sphaeropsis Sacc.
Stagonospora (Sacc.) Sacc.
** *Stauronema* (Sacc.) H. Sydow spp.

** cf. *Tetranacrium graminaum* Hudson & Sutton
Tiarosporella graminis (Pirozynski & Shoemaker) Nag Raj var *karoo* Sutton & Marasas
** *Tryblidiopycnis* Höhnel sp.

** *Urohendersonia platanoia* Speg.

MYCELIA STERILIA (AGONOMYCETES)

Papulospora Preuss sp.
Rhizoctonia D.C.sp.
Sclerotium rolfsii Sacc.

ZYGOMYCETES

Cunninghamella echinulata (Thaxter) Thaxter
Mortierella Coemans spp.
Mucor Michell ex Fr. spp.
Rhizopus stolonifer (Ehrenb.:Fr.) Vuill. var *stolonifer*
Rhizomucor (Lucet & Constantin) Wehmer ex Vuill. spp.

ASCOMYCETES

Ascotricha Berk. sp.
Ceratocystis Ell. & Halst. sp.
Chaetomium Kunze spp.
Leptosphaeria Ces. & de Not. spp.
Leptosphaerulina australis McAlp.
L. briosiana (Poll.) Graham & Luttrell
* *L. chartarum* C. Roux
Mycosphaerella tassiana (de Not) Johanson
Mycosphaerella Johanson spp.
Ophiobolus Riess sp.
Pezizales
** *Platyspora permunda* (Cke.) Wehm. = *Comoclathris* Clem.
Pleospora herbarum (Pers. ex Fr.) Rabenh.
Pleospora Rabenh. ex Ces. & de Not.
Saccobolus minimus Vel.
Sordaria fimicola (Rob.) Ces. & de Not.

MYXOMYCETES

Didymium Schrader
Physarum cinereum (Batch.) Pers.
cf. *Reticularia* Bull.
Stemonitis cf. *smithii* Macbr.

TABLE 7 The Ascomycetes, Zygomycetes, Mycelia Sterilia, Myxomycetes and unknown fungi as a percentage of the samples for 1978 to 1981.

	Litter POINT A 1978/79	Tribulus terrestris	Litter POINT B	Tribulus terrestris	Litter POINT C	Tribulus terrestris	Litter CAMP A 1979/80	Tribulus terrestris	Felicia muricata	Galenia procumbens	Galenia sarcophylla	Lycium cinereum	Cynodon incompletus	Eragrostis lehmanni	Litter CAMP B 1979/80	Tribulus terrestris	Felicia muricata	Galenia procumbens	Galenia sarcophylla	Lycium cinereum	Cynodon incompletus	Eragrostis lehmanniana	Litter CAMP A 1980/81	Tribulus terrestris	Felicia muricata	Galenia procumbens	Galenia sarcophylla	Lycium cinereum	Cynodon incompletus	Eragrostis lehmanniana	Litter CAMP B 1980/81	Tribulus terrestris	Felicia muricata	Galenia procumbens	Galenia sarcophylla	Lycium cinereum	Cynodon incompletus	Eragrostis lehmanniana
ASCOMYCETES																																						
Ceratocystis																																						
Chaetomium		0,7		0,1		0,5		0,7				0,7				0,4	1,9	0,4	0,4	0,4		0,3	0,2	0,4	0,5	0,4						0,1							
Leptosphaeria								3,0						1,1								2,4																
Leptosphaerulina		3,6	27,1	2,2	30,8	2,1	32,2	1,1	5,6		33,7	33,3	6,8	35,6	44,4	10,4	1,9	21,5	13,3	6,7	34,1	33,3	13,0	2,5	10,6	18,6	22,6	3,7	10,3	17,6	6,2	2,5	3,9	12,5	35,2	1,8	7,3	14,0	4,6
Mycosphaerella spp.			0,2		0,5		1,2				0,7	1,9		10,0	1,4			0,4	0,4	10,0	2,6			0,9	0,7	0,8	2,5		15,0	9,3	9,2	1,2	0,2	0,4	1,4	0,1	12,9	0,4	0,1
Ophiobolus circii				0,1	0,1																									0,1								
Platyspora permunda			0,3		0,3																				0,3		0,1		0,1									
Pleospora herbarum													0,4																	0,1				0,1			0,1	0,1
Pleospora sp.		0,1																0,4										0,1	0,1									
Saccobolus sp.		0,4		0,3		0,7																0,7	0,3							0,1						0,1		
Sordaria fimicola				0,1				1,9				1,1											0,1			0,1	0,7				0,1							
ZYGOMYCETES																																						
Cunninghamella sp.		0,2		0,1				0,7				0,7			0,4								0,1							0,1				0,2				
Mortierella spp.								0,4					0,7										2,1	0,7	0,2	0,2	1,1	0,1	0,2		1,6				0,1		0,8	0,1
Mucor spp.		1,9	0,2	1,6		2,5																	1,0	0,3	0,3	0,2		0,1	0,1	0,2	1,9	0,1	0,1		0,1		0,1	0,1
Rhizopus stolonifer			0,1						6,7					0,7			1,5	0,7	5,6		0,4	1,5	5,2	2,4	0,1	5,0	0,7	1,8	3,6	2,2	1,2	0,1	0,1	2,5	0,5	0,9	1,6
Rhizomucor sp.																												0,1						0,1		0,1		
MYCELIA STERILIA																																						
Papulospora sp.																							0,1															
Rhizoctonia spp.		2,6		1,5		5,9																	11,9	1,6	0,1	0,7	0,4		0,4	0,1	12,7	0,2			0,3			0,1
Sclerotium rolfsii																							0,2					0,1			0,5						0,1	
MYXOMYCETES								0,4						1,1									1,2		0,9		0,1				1,8	0,1	0,3				0,1	
BASIDIOMYCETES													0,4										0,1															
Agaricales																																						
Coprinus spp.																																						
Aphyllophorales																																						
Puccinia graminis																							0,4					1,5	5,0	0,3	0,1					0,2	3,6	0,1
Ustilaginales																									0,8												0,1	
UNKNOWN																																						
Ascomycetes		4,9	0,5	4,3	0,5	3,2	0,3	0,7			3,0			2,6	1,5	0,7				0,7	0,7	5,8	2,2		0,1	0,2	0,1	0,3	0,6				0,4	0,3	0,2	0,3	1,0	2,7
Discomycetes																							0,1								0,1							
Coelomycetes																											0,1					0,1						
Coelo no. 1																									0,1			0,1	0,1	0,2			0,4		0,1			1,2
Coelo no. 2																									0,2								0,5		0,1	0,1	0,4	5,0
Coelo no. 3																														0,1								
Coelo no. 4																									0,1									0,1				
Coelo no. 5																											2,0									2,5		
Coelo no. 6		0,3		0,3		0,3																												0,1				
Mycelia Sterilia																																						

TABLE 8 The Hyphomycetes as a percentage of the samples for 1978 to 1981.

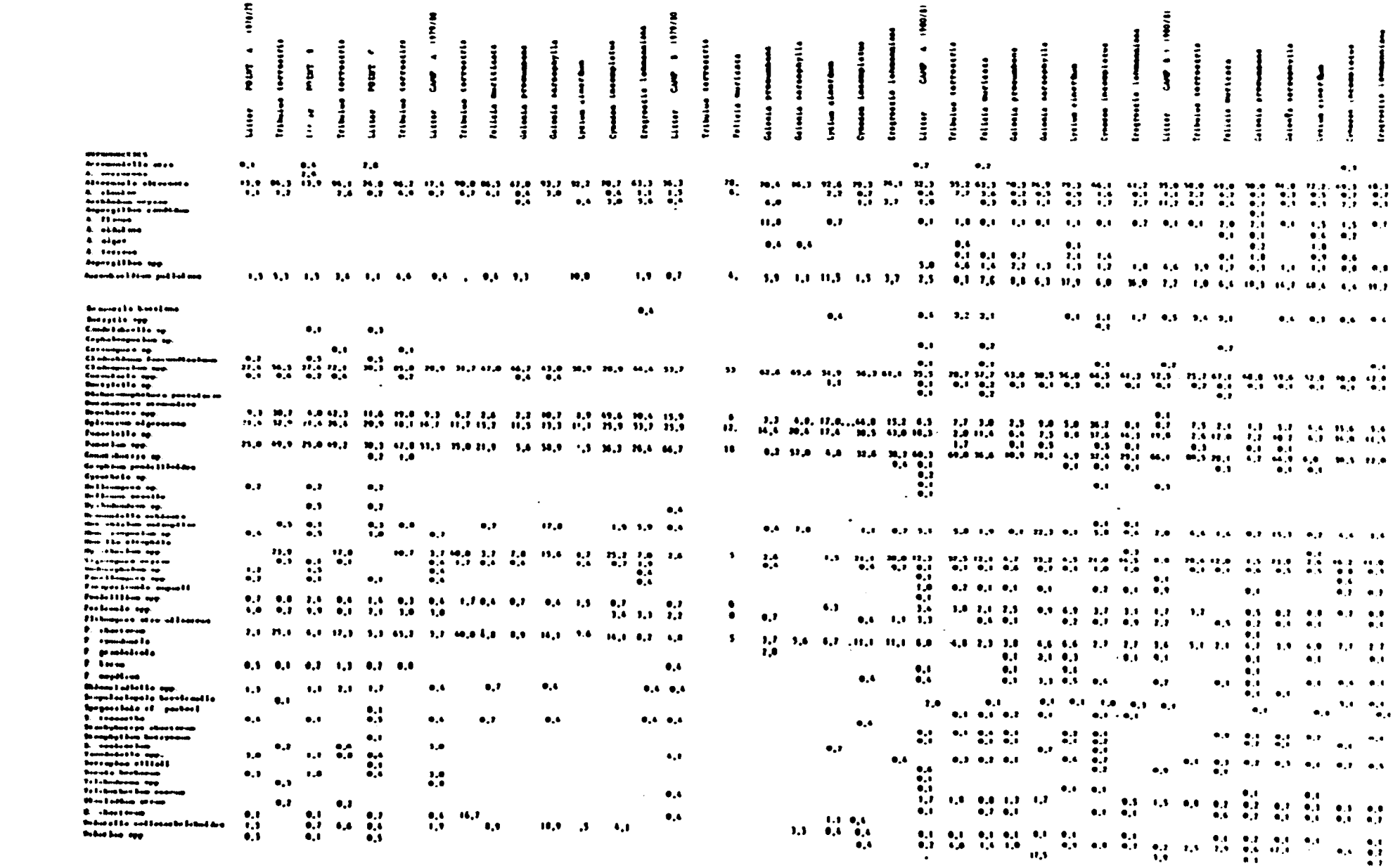
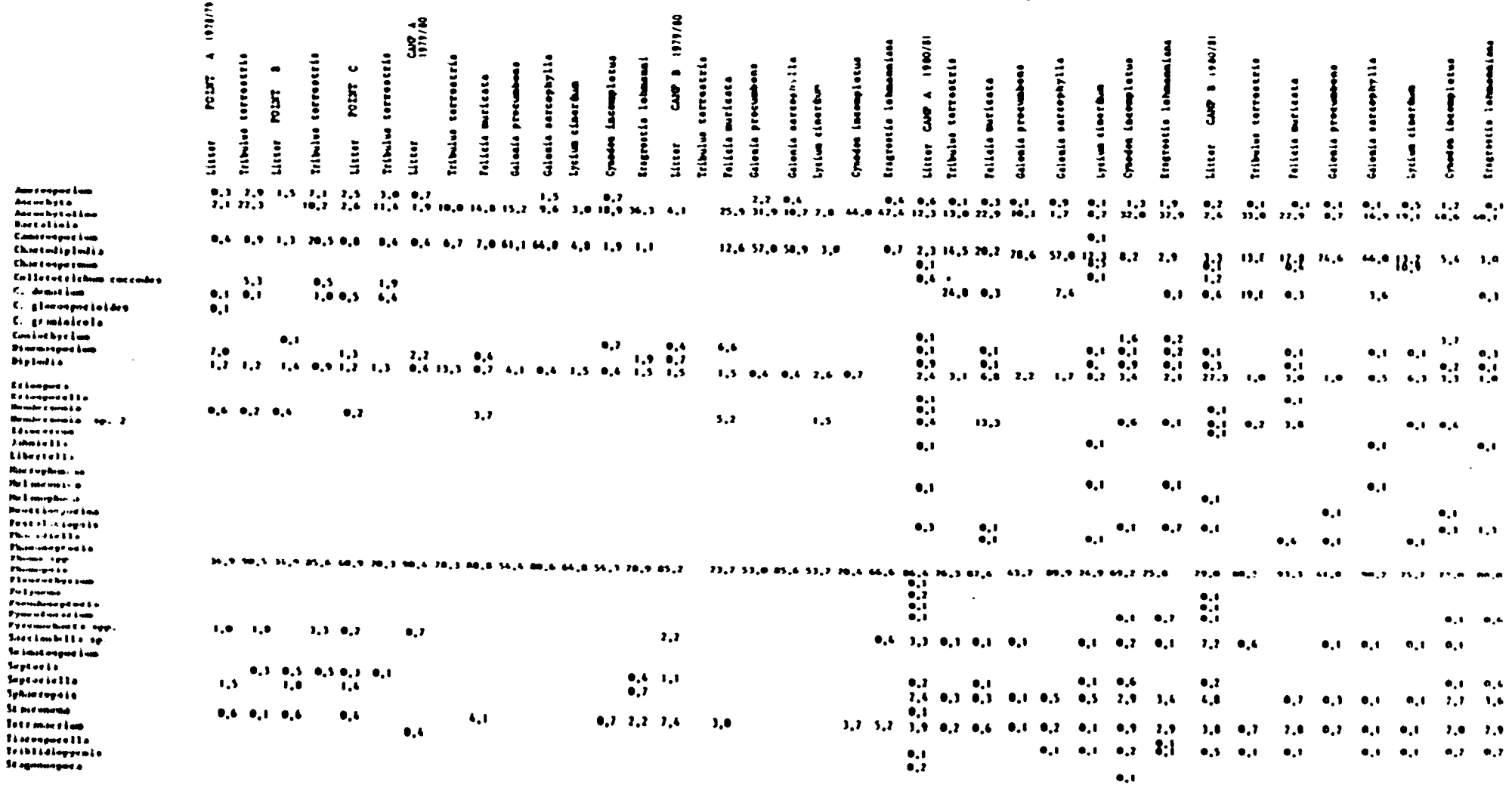


TABLE 9 The Coelomycetes as a percentage of the samples for 1978 to 1981.



REFERENCES

ACOCKS,J.P.H. (1975). Veld types of South Africa. *Memoirs of the Botanical Survey of South Africa* 40, 1 - 128.

ACOCKS,J.P.H. (1979). The flora that matched the fauna. *Bothalia* 12, 673 - 709.

ANDERSON,A.M. & McLACHLAN, I.R. (1976). The plant record in the dwyka and ecca series (Permian) of the south-western half of the Great Karroo Basin, South Africa. *Palaeotologia Africana* 19, 31 - 42.

BEZUIDENHOUT,HENRIETTA. (1977). 'n Ondersoek van die Hyphomycetes geassosieer met Cenchrus ciliaris L. M.Sc. Thesis, Rand Afrikaans University, Johannesburg, S.A.

CLUVER, M.A. (1978). *Fossil reptiles of the South African Karoo.* Cape Town : South African Museum.

COMMONWEALTH MYCOLOGICAL INSTITUTE (1983). *Plant Pathologist's Pocketbook,* 2 nd edn. Kew : Commonwealth Agricultural Bureaux.

CRAWLEY,W.E. & WOOLFORD,M.W. (1965). Predicting facial eczema danger periods. *Proceedings of the Ruakura Farmers' Conference Week* 15 - 21.

DICKINSON,C.H. (1967). Fungal colonization of Pisum leaves. *Canadian Journal of Botany* 45, 915 - 927.

DI MENNA,M.E. & PARLE,J.N. (1970). Moulds of leaves of perrenial rye-grass and clover. *New Zealand Journal of Agricultural Science* 13, 51 - 68.

DI MENNA,MARGARET E., CAMPBELL,J. & MORTIMER,P.H. (1970). Sporidesmin

production and sporulation by Pithomyces chartarum. *Journal of General Microbiology* 61, 87 - 96.

DOIDGE,ETHEL M. (1950). The South African Fungi and Lichens to the end of 1945. *Bothalia* 5, 1 - 1094.

DU TOIT,A.L. (1954). *The Geology of South Africa.* Edinburgh : Oliver & Boyd.

EICKER,A. (1973). The mycoflora of Eucalyptus maculata leaf litter. *Soil Biology and Biochemistry* 5, 441 - 448.

EICKER,A. (1976). The non-parasitic mycoflora of the phylloplane and litter of Panicum coloratum. *Transactions of the British Mycological Society* 67, 275 - 281.

HERING,T.F. (1965). Succession of fungi in the litter of a lake district oakwood. *Transactions of the British Mycological Society* 48, 391 - 408.

HOGG, BARBARA M. & HUDSON,H.J. (1966). Microfungi on leaves of Fagus sylvatica. *Transactions of the British Mycological Society* 49, 185 - 192.

HUDSON,H.J. (1968). The ecology of fungi on plant remains above the soil. *New Phytologist* 67, 837 - 874.

HUTCHEON,D. (1886). *Report of the Veterinary Surgeon.* Cape Town: Cape of Good Hope, G14 - 1887.

KELLERMAN,T.S.,VAN DER WESTHUIZEN,G.C.A., COETZER,J.A.W., ROUX, CECILIA, MARASAS,W.F.O., MINNE,J.A.,BATH,G.F. & BASSON, P.A. (1980). Photosensitivity in South Africa.II. The

experimental production of the ovine hepatogenous photosentivity disease geeldikkop (Tribulosis ovis) by the simultaneous ingestion of Tribulus terrestris plants and cultures of Pithomyces chartarum containing the mycotoxin sporidesmin. *Onderstepoort Journal of Veterinary Research* 47, 231 - 261.

LATCH,G.C.M. & McKENZIE,E.H.C. (1977). Fungal flora of rye grass swards in Wales. *Transactions of the British Mycological Society* 68, 181 - 184.

LINDSEY,B.I. & PUGH,G.J.F. (1976) Succession of microfungi on attached leaves of Hippophaë rhamnoides. *Transactions of the British Mycological Society* 67,61 - 67.

MARASAS,W.F.O. & SCHUMANN, INGRID H. (1972).The genus Pithomyces in South Africa. *Bothalia* 10, 509 - 516.

MARASAS,W.F.O., ADELAAR,T.F.,KELLERMAN,T.S.,MINNE,J.A., VAN RENSBURG, I.B.J. & BURROUGHS,G.W. (1972). First report of facial eczema in sheep in South Africa. *Onderstepoort Journal of Veterinary Research* 39, 107 - 112.

McKENZIE,E.H.C.& HUDSON,H.J.(1976). Mycoflora of rust-infected and non-infected plant material during decay. *Transactions of the British Mycological Society* 66, 223 - 238.

McLACHLAN,I.R. & ANDERSON,A.M. (1977). Fossil insect wings from the early Permian white band formation, South Africa.

Palaeontologia Africana 20, 83 - 86.

MÖHR,J.D. (1982). The Karoo caterpillar Loxostege frustalis Zeller (Lepidoptera:Pyralidae) in relation to its host plants and natural enemies. Ph.D.Thesis, Rhodes University, Grahamstown,S.A.

NIENABER,P.J. (1963). *Dictionary of South African Place Names*. Cape Town:South African Book Centre.

PAPENDORF, M.C. & JOOSTE,W.J. (1974). The mycoflora of wheat field debris, Part I. *Bothalia* 11, 201 - 207.

PRICE,P.W. (1975). *Insect Ecology*. New York:John Wiley & Sons.

PUGH,G.J.F.(1958). Leaf litter fungi found on Carex paniculata L. *Transactions of the British Mycological Society* 41, 185 - 195.

PUGH,G.J.F. & MULDER,J.L. (1971). Mycoflora associated with Typha latifolia. *Transactions of the British Mycological Society* 57, 273 - 282.

ROUX,CECILIA. (1977). 'n Studie van die voorkoms, verspreiding en morfologie van Pithomyces chartarum (Berk. & Curt.) M.B. Ellis in Suid-Afrika en sommige aspekte van sy fisiologie. M.Sc. Thesis, Rand Afrikaans University, Johannesburg,S.A.

RUSCOE,Q.W. (1971). Mycoflora of living and dead leaves of Nothofagus truncata. *Transactions of the British Mycological Society* 56, 463 - 474.

SOUTHWOOD,T.R.E.(1977). Habitat, the templet for ecological strategies? *Journal of Animal Ecology* 46, 337 - 365.

SUTTON, B.C. (1980). *The Coelomycetes*. Kew, Commonwealth Mycological Institute.

THEILER,A. (1918). Geeldikkop in sheep (Tribulosis ovium). *Report on Veterinary Research*, Union of South Africa 7 & 8, 1 - 55.

THORNTON,R.H. & PERCIVAL,J.E. (1959). A hepatotoxin from Sporidesmium bakeri capable of producing facial eczema in sheep. *Nature, London*. 183, 63.

THORNTON,R.H. & ROSS,D.J. (1959). The isolation and cultivation of some fungi from soils and pastures associated with facial eczema disease in sheep. *New Zealand Journal of Agricultural Research* 2, 1002 - 1016.

VAN DER MERWE, W.J.J., EICKER,A.,MARASAS,W.F.O. & KELLERMAN,T.S. (1979). Aerospora of an Eragrostis curvula pasture in South Africa. *Onderstepoort Journal of Veterinary Research* 46, 19 - 25.

VAN TONDER,E.M.,BASSON,P.A. & VAN RENSBURG,I.B.J. (1972). 'Geeldikkop' experimentally induced by feeding Tribulus terrestris L. (Zygophyllaceae). *Journal of the South African Veterinary Association* 43, 363 - 375.

WATT, J.M. & BREYER-BRANDWIJK, MARIA GERDINA (1962). *The Medicinal and Poisonous Plants of Southern and Eastern Africa. Being an Account of their Medicinal and Other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal.* Edinburgh: Livingstone.

SECTION II

LEPTOSPHAERULINA CHARTARUM SP. NOV.,

THE TELEOMORPH

OF

PITHOMYCES CHARTARUM

CONTENTS

ABSTRACT

Leptosphaerulina chartarum sp.nov., the teleomorph of *Pithomyces* is described from various substrata in a natural Karoo pasture in South Africa. The ascospores are similar in morphology to the conidia, but are smooth and hyaline to light brown. This is the first anamorph reported for *Leptosphaerulina* and likewise the first teleomorph reported for *Pithomyces*.

INTRODUCTION

As early as 1918 Theiler, with the botanical assistance of I. B. Pole Evans, proved that Tribulus terrestris L., a pioneer plant (caltrop U.S.A.; three cornered jack, Australia; Mexican sandbur) (Watt & Breyer-Brandwijk, 1962) was responsible for ' geeldikkop ' (Eng. yellow thick head) Tribulosis ovis of sheep. Theiler (1918) noted the presence of a fungus, Colletotrichum sp. on the toxic plants. Meanwhile, in New Zealand, a similar disease called facial eczema, was noted in the 1908 annual report of the Department of Agriculture (Filmer, 1958). Percival & Thornton (1958) showed with their ' beaker test' that pastures which were toxic contained mycelium of a fungus, later identified by CMI as Sporidesmium bakeri Syd., implicated in this disease. Ellis (1960) redescribed this fungus naming it Pithomyces chartarum (Berk. & Curt.) M.B. Ellis. Facial eczema of sheep was first diagnosed in South Africa during the outbreak at Humansdorp where P. chartarum was isolated from the pasture (Marasas et al.,1972). Studies on the morphology of the genus Pithomyces in South Africa followed (Marasas & Schumann, 1972).

On account of the similarities between facial eczema and ' geeldikkop ' which occurs in the arid Karoo region, fungi, especially P. chartarum occurring on T. terrestris were studied. The first isolates of P. chartarum from T. terrestris in the Karoo were collected during 1976 from Aberdeen, Cape Province (Roux, 1977). Since then this fungus has been found to be present all over southern Africa, both on plants and in the soil (C. Roux, unpubl.), indicating it to be truly

indigenous. During serious outbreaks of the disease up to half a million sheep can be affected (Steyn, 1949). Pure cultures of toxigenic isolates of *P. chartarum* together with *T. terrestris*, to provide phylloerythrin, were given to sheep under controlled conditions thereby reproducing 'geeldikkop ' (Kellerman *et al.*, 1980).

From 1978 to 1982 a survey was undertaken to study the mycoflora of a natural pasture in the Karoo. The main aim was to determine the levels at which *P. chartarum* was present, especially at times when ' geeldikkop ' could occur. During this survey several new records for South Africa and undescribed fungi were found, the most important of these being *Leptosphaerulina chartarum* sp.nov., the teleomorph of *P. chartarum*.

DESCRIPTION OF THE FUNGUS

The presence of fungi was noted on seven plants and litter after being plated out directly on potato agar (CMI, 1983) plus 125 mg Albamycin-T/l (UpJohn). The plates were incubated for 7 d at 24°C under mixed uv and daylight fluorescent tubes on a 12h/d cycle. Isolations were made to verify identifications and cultures of *P. chartarum* were assayed for sporidesmin toxin production (Marasas *et al.*, 1972) on semi-synthetic broth (Di Menna, Campbell & Mortimer, 1970).

It was noted that in very rare cases the Leptosphaerulina ascostromata in the leaves were apparently ' parasitized ' by P. chartarum. In the ostiole of the ascostromata, where one would expect the asci to emerge, a number of conidia of P. chartarum could be found. P. chartarum did not grow on the general surface of the leaves in these cases, but was localized on the ascostromata and associated with them. The ' parasitized' ascostromata were observed on leaves of Galenia procumbens L.F., T. terrestris and Cynodon incompletus Nees. The anamorph of Cochliobolus nodulosus Luttrell has developed directly from the teleomorph on seed of Sorghum vulgare Beauv. in a similar manner (R.Y.Anelich, pers. comm.).

Conidia isolated from one of these ascostromata, gave rise to a culture in which ascostromata developed. This isolate, Gp 2/24 A1, produced sporidesmin at a level of 10 mg/ l, which is average for toxigenic cultures of P. chartarum obtained from the Karoo. This culture was subsequently lyophilized and revived at intervals to test its stability. Initially all single ascospore cultures gave rise to the anamorph only. However, after some time it was found that incubation temperature affected the physiology of the fungus. Thus, single ascospore cultures grown at 20^{o} on 1,5% malt extract agar (CMI, 1983) consistently gave rise to ascostromata and discharged ascospores actively. However, at 24^{o} on the same medium little active spore discharge took place and the anamorph was formed. When the conidial cultures were cultured together at either

temperature, no ascostromata developed, which suggests a heterothallic condition in P. chartarum. In cultures grown at 24°, ascostromata which did not readily discharge their spores, resembled those initially found on the leaves where the anamorph was formed in the ostiole (Fig. 1).

Leptosphaerulina chartarum sp.nov.
Anamorphosis Pithomyces chartarum (Berk. & Curt.) M.B.Ellis

In cultura in extracto agari 1,5% ad 24° luce: colonia lanata vel adpressea, atro-olivacea. Mycelia atro-olivacea-brunnea, septata. Ascostromata separata, atro-olivaceo-brunnea, globosa, ostiolo amplo, immersa, superficialia, aparaphysata. Asci 5 - 7, brevis ovati maturate longiscentes, bitunicati 100 - 150 x 60 - 100 µm; ascosporae 8 per ascum, dictyosporae, plerumque, 3 ti transversalibus et 1 longi- dinalibus, septatae, constrictae, hyalinae vel pallide brunneae, laeves, late ellipsoidales 23 (25) 27 x 7 (8) 12 µm.

In folii mycelium immersum atro-brunneam, septata. Ascostromata separata, immersa ad erumpentia, globosa, ostiola emplo, aparaphysata. Ascosporae, hyalinae vel pallide brunneae.

Ex folii Galenia procumbens L.F., Grootfontein, Middelburg, C.P. Austroafricanum, 1981, A. Barnhoorn. C. Roux Gp 2/24 A1, PREM 47900 holotypus.

In culture on 1.5% MEA at 24° with uv and daylight fluorescent tubes on a 12 h/d cycle: colony woolly to appressed, dark olive. Mycelium dark olive brown, septate. Ascostromata separate, dark olive brown, globose, large ostiole, immersed and superficial in medium, aparaphysate. Asci 5 - 7, short ovate becoming longer with age, bitunicate 100 - 150 x 60 x 100 µm. Ascospores 8 per ascus, dictyosporous, usually three transverse and one longitudinal septum, constricted at septa, hyaline to light brown, smooth, broadly ellipsoidal, 23 (25) 27 x 7 (8) 12 µm.

On leaves mycelium immersed, dark brown. Ascostromata separate, immersed to erumpent, globose, ostiole large.

DISCUSSION

In order to verify the identity of the teleomorph, the ascostromata were studied at various stages to determine their developmental characteristics. Asci and interthecial cells (Wehmeyer, 1955) or strands of interascular stromatal tissue (Luttrell, 1973) were distinguished in the developing ascostromata. A small number of asci, 5 - 7, usually developed in sequence from a central point (Fig. 2). Asci are short ovate, elongating during maturation. In very rare cases a dome-like structure (Graham & Luttrell, 1961) could be seen at the apex of the ascus (Fig. 3) which is typical of the genus Leptosphaerulina McAlp. Dendritic ascostromata developed in culture where ascospore release could not take place satisfactorily. There

are indications that asci lodged in the ostiole, developed into ascostromata which in turn gave rise to another order of ascostromata (Fig. 5, 6). These dendritic ascostromata were never observed on the leaves. Ascostromata in artificial culture were produced in the aerial mycelium, on and in the agar substrate.

Septation of propagules in both states is very similar (Fig. 4, 7) and their size correspond to a certain extent. Ellis (1960) gave the range for P. chartarum as 18 - 29 x 10 - 17 µm while Marasas & Schumann (1972) gave the following measurements: 14 (20) 36 x 8 (12) 21 µm with septation 0 (3) 5 x 0 (2) 3 (transverse x longitudinal). Roux (1977) obtained the following mode measurements at 20° on 1,5 % MEA: (24) x (13) µm and at 24° on the same medium (21) and (12) µm while septation at both these conditions was 2 (3) 6 x 0 (2) 3. Measurements obtained for the conidia of cultures of L. chartarum were at 20° : 20.5 (23) 25.5 x 9.5 (11.7) 14.0 µm and septation (3) 4 x (1) 2. The ascospores which were not released from the asci measured 31 (30) 35 x 10 (12) µm while the transverse septa can increase to five. These were the same as those obtained for germinating ascospores. The ascospores were smooth and hyaline, becoming dark long after ejaculation, and possess a distinct sheath (Von Arx & Müller, 1975) which sometimes diminished although it may remain intact until germination takes place (Fig. 4).

Ascospores of L. briosiana (Poll.) Graham & Luttrell and of L. trifolii (Rost.) Petrak, a species associated with leaf spots on legumes

(Graham & Luttrell, 1961) are larger than those of L. chartarum but septation bears some resemblance. L. chartarum was never associated with any spots or lesions and no representatives of the Leguminosae were present in the veld where it was collected.

Ascospores of Dictyosphaerella andropogonis Doidge (1922) were compared from the holotype PREM 1214 which consisted of leaves of Cymbopogon validus Stapf ex Burtt Davy. This fungus has not been collected since originally described. Petrak (1927) discussed the taxonomic problems concerning this fungus and stated that it was best interpreted as a Pleospora and named it Pleospora doidgeae Petrak. Wehmeyer (1961) noted that there is a difference concerning the ascomata which are paraphysate and the asci grouped together in separate bundles in the ascostromatic circumstructure, describing it as ' having a pseudosphaeriaceous appearance ' . The ascospores are morphologically similar to L. chartarum, the only difference being the acutely pointed apices in P. doidgeae. The spore dimensions are very similar.

Conidia were more abundant under arid hot conditions while the discovery of an ascomycetous state could explain the ability of the fungus to survive during winter and produce a flush of conidia as soon as the weather improved. Such a cycle would coincide with the first flush of growth of T. terrestris, a pioneer plant which appears after the first summer rain has fallen in this semi-desert environment.

Cultures of L. chartarum can be obtained from the National Collection

of Fungi, Culture Collection, Private Bag x 134, Pretoria 0001 South Africa.

I am especially grateful to the Director, Karoo Region, Dr. P.W. Roux and to Mr. A. Barnhoorn who collected samples, my colleagues in the Mycological Research Unit for unstinting support, Dr. D.J. B. Killick, Botanical Research Institute for help with the Latin diagnosis and Professor K.T. van Warmelo , Rand Afrikaans University, for his faith in me and encouragement. This work forms part of a dissertation for the Ph. D. degree at the Rand Afrikaans University.

Fig. 1 An ascostroma of Leptosphaerulina chartarum with asci protruding laterally through its wall and Pithomyces chartarum conidia being produced in the ostiole from and from two separate asci and laterally (Bar = 50 µm)

Fig. 2 Asci from one ascostroma of L. chartarum stained with Meltzer's Iodine (Bar = 50 µm)

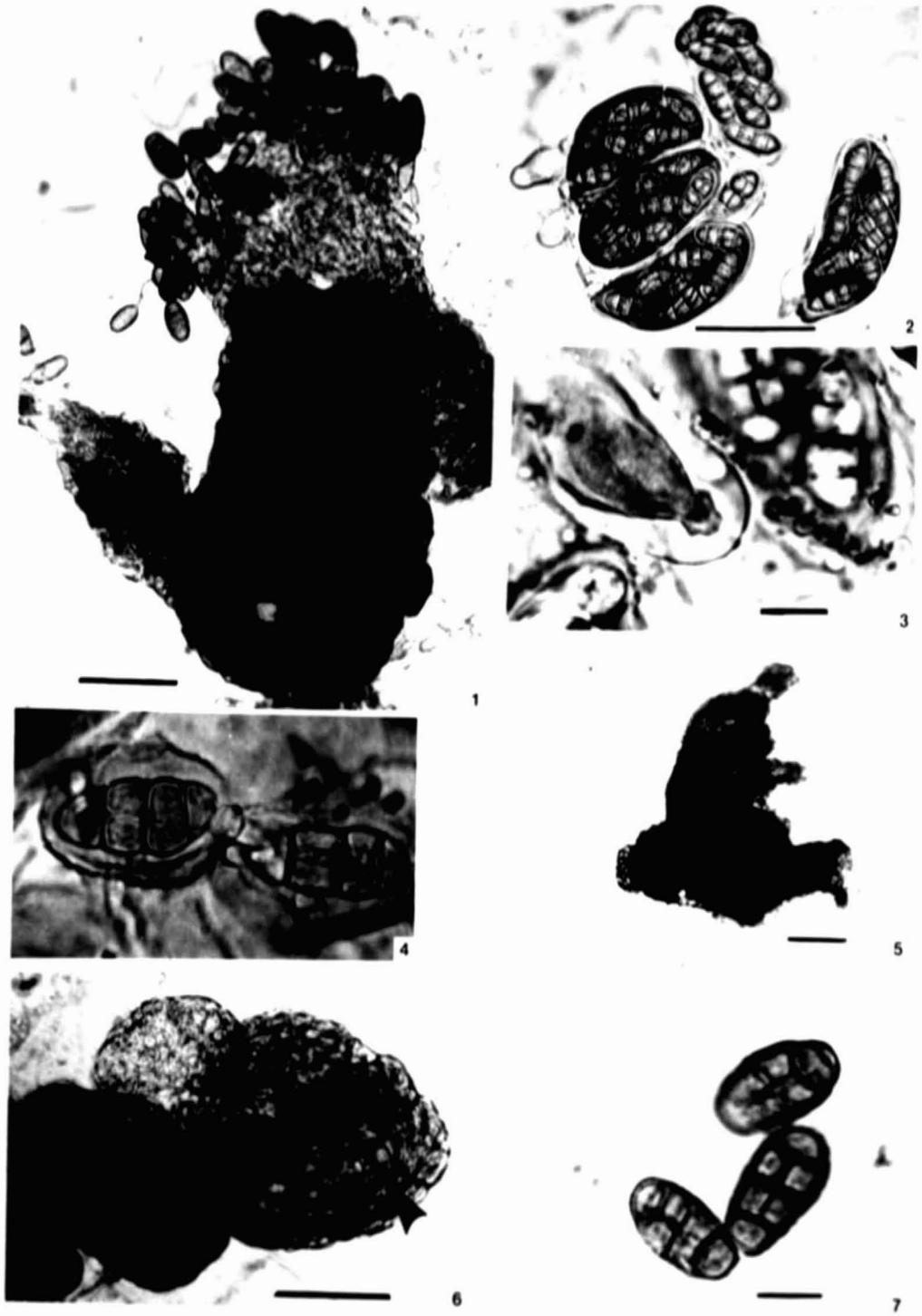
Fig. 3 Dome-like apical structure of an immature ascus of L. chartarum stained with Meltzer's Iodine (Bar = 10 µm)

Fig. 4 Gelatinous sheaths on two germinating ascospores of L. chartarum grown on 1,5% MEA, 20° (Bar = 10 µm)

Fig. 5 A young dendritic ascostroma of L. chartarum grown on 1,5% MEA, 20° (Bar = 50 µm)

Fig. 6 Detail of a dendritic ascostroma with encased ascus (arrowed) (Bar = 50 µm)

Fig. 7 Conidia of P. chartarum originating from a single ascospore culture (Bar = 10 µm)



REFERENCES

COMMONWEALTH MYCOLOGICAL INSTITUTE (1983). *Plant Pathologist's Pocketbook*, 2nd edn. Kew: Commonwealth Agricultural Bureaux.

DI MENNA, MARGARET E., CAMPBELL,J & MORTIMER,P.H. (1970). Sporidesmin production and sporulation in Pithomyces chartarum. *Journal of general Microbiology* 61,87 - 96.

DOIDGE,ETHEL M. (1922). South African ascomycetes in the National Herbarium, Part I, No. 51 - 85. *Bothalia* 1, 66.

ELLIS, M.B. (1960). Dematiaceous Hyphomycetes : I. *Mycological Papers* (CMI) 76, 13.

FILMER, J.F. (1958). The story of facial eczema research. *New Zealand Journal of Agriculture* 97, 202 - 209.

GRAHAM, J.H. & LUTTRELL, E.S. (1961). Species of Leptosphaerulina on forage plants. *Phytopathology* 51, 680 - 693.

KELLERMAN, T.S., VAN DER WESTHUIZEN,G.C.A., COETZER,J.A.W., ROUX, CECILIA, MARASAS,W.F.O., MINNÉ, J.A., BATH, G.F. & BASSON,P.A. (1980). Photosensitivity in South Africa. II The experimental production of the ovine hepatogenous photosensitivity disease geeldikkop (Tribulosis ovis) by the simultaneous ingestion of Tribulus terrestris plants and cultures of Pithomyces chartarum containing the mycotoxin sporidesmin. *Onderstepoort Journal of Veterinary Research* 47, 231 - 261.

LUTTRELL,E.S. (1973). Ascomycotina, Loculoascomycetes. In *The Fungi*, vol. IV A (ed. G.C. Ainsworth, F.K. Sparrow & A.S. Sussman), pp 135 - 219. Cambridge University Press.

MARASAS,W.F.O. & SCHUMANN, INGRID H. (1972). The genus Pithomyces in South Africa. *Bothalia* 10, 509 - 516.

MARASAS,W.F.O., ADELAAR,T.F., KELLERMAN,T.S., MINNÉ, J.A., VAN RENSBURG,I.B.J. & BURROUGHS, G.W. (1972). First report of facial eczema in sheep in South Africa. *Onderstepoort Journal of Veterinary Research* 39, 107 - 112.

PERCIVAL,J.C. & THORNTON,R.H. (1958). Relationship between the presence of fungal spores and a test for hepatotoxic grass. *Nature, London* 182, 1095 - 1096.

PETRAK,F. (1927). Mykologische Notizen : IX. *Annales mycologici* 25, 293 - 295.

ROUX, CECILIA (1977). 'n Studie van die voorkoms, verspreiding en morfologie van Pithomyces chartarum (Berk. & Curt.) M.B. Ellis in Suid-Afrika en sommige aspekte van sy fisiologie. M.Sc. Thesis, Rand Afrikaans University, Johannesburg, S.A.

STEYN,D.W. (1949). *Vergiftiging van mens en dier.* Cape Town : Van Schaik.

THEILER, A. (1918). Geeldikkop in sheep (Tribulosis ovium). *Report on Veterinary Research, Union of South Africa* 7 and 8, 1 - 55.

VON ARX, J.A. & MÜLLER, E. (1975). A re-evaluation of the bitunicate Ascomycetes with keys to families and genera. *Studies in Mycology* (CBS) 9, 7 - 9 , 89.

WATT, J.M. & BREYER-BRANDWIJK, MARIA GERDINA (1962). *The Medicinal and Poisonous Plants of Southern and Eastern Africa. Being an Account of their Medicinal and Other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal.* Edinburgh :Livingstone.

WEHMEYER,L.E. (1955). The development of the ascocarp in Pseudoplea gaeumannii. *Mycologia* 47, 163 - 176.

WEHMEYER,L.E. (1961). *A World Monograph of Pleospora and its Segregates*. Ann Arbor : University of Michigan Press

(Received for publication 6 June 1985)

Transactions of the British Mycological Society

S E C T I O N I I I

MORPHOLOGY AND CONIDIOGENESIS

IN THREE SELECTED

COELOMYCETES

CONTENTS

ABSTRACT

The study of the conidiogenesis of three coelomycetes confirmed their holoblastic development using light andtransmission electron microscopy. The conidiogenous cell in Urohendersonia platensis Speg. and Bartalinia robillardoides Tassi has been altered to allow the production of a maximum of three conidia only. This necessitated the description of a new mode of conidiogenesis, viz. thysanoblastic. The conidiogenous apparatus in these two species and Tiarosporella graminis (Pirozynski & Shoemaker) Nag Raj var karoo Sutton & Marasas was studied and certain discrepancies with the existing literature were recorded. The ontogeny of the conidiomata and conidiogenous locules in culture was examined.

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Up to fairly recently the classification of the Deuteromycetes was based on the morphology of the conidia and mature fructifications. The criteria eventually proved inadequate which led to the process of conidiogenesis receiving more and more attention, especially in the classification of the Hyphomycetes. Similar investigations in the Coelomycetes have depended very heavily on the data obtained from the Hyphomycetes and furthermore, very little is known regarding the ontogeny of the conidioma. Identification of Coelomycetes thus continued to rely on mature morphology until 1980 when details of conidiogenesis and differences in conidiomatal structure were used as a basis for a key (Sutton, 1980).

The prevalence of the Coelomycetes in the Karoo fungi and the associated difficulties of identification made fundamental knowledge of their morphology and conidiogenesis an absolute requirement. This in turn developed into an in-depth study of selected species. The genera selected, Urohendersonia, Tiarosporella and Bartalinia, had some characteristics in common, viz. all possess conidial appendages and conidiogenesis is holoblastic (Sutton, 1980). The results of this study are presented here.

LITERATURE REVIEW

Conidiogenesis has been increasingly used in Deuteromycetes systematics since Hughes (1953) classified some Hyphomycetes according to the different methods by which conidia grew before, during and after they are produced. Since then others(Subramanian, 1962; Tubaki, 1963; Barron, 1968; Ellis, 1971) have used this criterion to classify the Hyphomycetes while the first system using conidiogenesis as a basis for the taxonomy of the Coelomycetes was proposed by Sutton (1980). Cole & Samson (1979) emphasized the importance of ultrastructural studies to elucidate conidiogenesis. Minter, Kirk & Sutton (1982; 1983) reassessed optical and electron microscopic observations and demonstrated that instead of discrete categories there was a continuous spectrum of developmental processes.

A simplified system of blastic and thallic conidiogenous processes was proposed at the first Kananaskis Conference (Kendrick, 1971). The blastic process was further subdivided into a holoblastic category, where all or both cell walls contributed to the formation of the next conidium and the enteroblastic category where only the inner wall layers of the conidiogenous cell gave rise to the next conidium.

Enteroblastic conidiogenesis was subdivided into two types; the tretic and phialidic. Ellis (1971) described the tretic process as one where

the conidium was formed by proliferation of the inner wall layers through a pore in the outer layers. The process of phialidic conidium ontogeny as it occurs in Hyphomycetes has been described in detail (Cole & Samson, 1979) as a process in which successive conidia arose from a constant conidiogenous locus on a phialide. The succession of scars left by the released conidia eventually formed a periclinal thickening at the apex of the phialide, with or without a prominent collarette, with a fixed growing point (Kendrick & Carmichael, 1973). An additional mechanism of conidiogenesis which was pertinent to the present study was annellidic ontogeny. In this case conidial secession takes place at a progressively higher or lower conidiogenous locus (Sutton & Sandhu, 1969; Kendrick, 1971) which would result in either an increase or decrease in length of the conidiogenous cell. The outer wall layers of the conidium were thus synthesized by the developing conidium and were not continuous with the outer wall layers of the conidiogenous cell. Nag Raj & Kendrick (1971) equated annellides to percurrent proliferations - a term first used by Luttrell (1963).

It was noted at the First Kananaskis Conference that there were differences in the interpretation of what had been seen with the light and electron microscopes (Kendrick, 1971). What would have been interpreted as ' porogenous ' by Luttrell (1963) was termed ' tretic ' conidiogenesis for Phoma herbarum Westend. by Boerema (1964).

Sutton & Sandhu (1969) studied conidium development and secession in three annellidic genera of the Coelomycetes and concluded that Phoma fumosa Ell. & Ev. displayed annellidic conidiogenesis. Marasas, Pauer & Boerema (1974) noted that the correct terminology for the ' budding ' observed in various Phoma spp. studied earlier was ' phialides ' according to Kananaskis I (Kendrick, 1971). Sutton (1980) stated that tretic conidiogenesis has not been confirmed in coelomycetes, although blastosporic, annellidic and phialidic conidiogenesis were well known.

Subramanian (Kendrick, 1971) confined the term ' conidiophore ' to the cell bearing the conidiogenous cell which in turn produced the conidium from the conidiogenous locus on the cell. Carroll & Carroll (1971) stated that the conidium ontogeny in Stemphylium botryosum was initially blastic and then became tretic. Subramanian added in the discussion that followed that conditions may change the conidiogenous process from blastic to tretic. Pirozynski added in the discussion that plastic cells were blastic whereas this could change later to become different when the wall became thick, non-elastic and fully differentiated. It was noted at the Kananaskis I Conference that there were differences in the interpretation of what had been seen with the light and electron microscopes. At Kananaskis II Madelin (1979) elaborated on the blastic and tretic conidium, but did not mention a single-layered wall producing conidia by tretic conidiogenesis.

The literature regarding the three selected Coelomycetes studied can be summarized as follows:

The genus Urohendersonia Speg.

The genus Urohendersonia Speg. was described from the Argentine with U. platensis Speg. as holotype and later monographed by Nag Raj & Kendrick (1971). Sutton, Ghaffar & Abbas (1972) amended the genus and Sutton (1980) provided a key.

The only collection of U. platensis until the isolations mentioned in Table 1 were obtained, was the original specimen. The description of the genus according to Sutton (1980) reads as follows:

The conidioma is an ostiolate pycnidium, amphigenous, separate, gregarious or confluent. Conidiophores absent. Conidiogenous cells holoblastic and determinate or annellidic and indeterminate, discrete, ampulliform to doliiform, pale brown to hyaline,smooth with 0 - 2 percurrent proliferations,formed from the inner cells of the pycnidial wall. Conidia pale brown, 3 euseptate, continuous or constricted, cylindrical, clavate or fusiform, apex obtuse. Base truncate often with a marginal frill, thin-walled,smooth or verruculose, occasionally guttulate, with an extracellular, gelatinous, unbranched, single, apical appendage.

The genus Tiarosporella Höhnel

The genus Tiarosporella was erected by Höhnel to accommodate some species of Neottiospora (Pirozynski & Shoemaker, 1971). T. graminis

Pirozynski & Shoemaker was emended by Nag Raj (1973) and a variety T. graminis var karoo described by Sutton & Marasas (1976) from the Karoo. Sutton (1980) provided a key to the known species.

The genus Tiarosporella was described by Sutton (1980) as follows:

Conidioma pycnidial, separate or linearly aggregated, globose, dark brown, unilocular, thick walled. Ostiole central, circular, papillate. Conidiophores absent. Conidiogenous cells holoblastic, determinate, discrete, lageniform, hyaline, smooth, eguttulate, cylindrical to fusiform, straight or slightly curved, with an apical gelatinous appendage originating by the splitting from the base of an envelope enclosing young conidia, finally dividing into 2 - 4 unbranched tentacles.

T. graminis var karoo Sutton & Marasas

The diagnostic character of this variety is the rostrate conidioma.

The genus Bartalinia Tassi

The genus Bartalinia Tassi was erected with type (holo) B. robillardoides Tassi. A recent description of the genus was given by Morgan-Jones, Nag Raj & Kendrick (1972) although it was not revised.

The description of the genus is as follows (Sutton, 1980) :

Conidioma pycnidial, separate, globose, dark brown, ostiolate, slightly papillate. Conidiophores absent. Conidiogenous cells holoblastic, annellidic, indeterminate, discrete, cylindrical to lageniform, smooth with 1 - 2 percurrent proliferations, formed from the inner cells of the pycnidial wall. Conidia hyaline to pale brown, 4 - euseptate, the penultimate basal cell longer than the rest, continuous,cylindrical to fusiform, straight or slightly bent, base truncate, apex conic, thin-walled but two median cells slightly thicker than median cells, eguttulate, with 2 - 3 apical cellular unbranched appendages and a single exogenous cellular unbranched appendage.

MATERIALS AND METHODS

MATERIALS

Isolates used in this study were mainly obtained from the Karoo while additional material from other localities was obtained from the PREM culture collection (Fig.1,Table 1).

MEDIA USED:

Potato Carrot Agar (PCA) ; 2% Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) all according to CMI (1983);
Carnation Leaf Agar (CLA) (Fisher et al., 1982) ;
PCA and carnation leaflets sterilized by gamma irradiation;
PCA and autoclaved filter paper strips and
Potato Carrot Broth (PCB) for mass cultures of Urohendersonia platensis only.

CULTURE CONDITIONS

Cultures grown on solid media were grown in disposable plastic petri dishes while liquid cultures were grown in Pyrex Erlenmeyer flasks. The same conditions were used for studies on conidiogenesis as were used for the initial isolation. These conditions were 24° C with combined near ultraviolet and daylight fluorescent illumination from a height of 50 cm on a 12 h/d cycle.

METHODS

LIGHT MICROSCOPY

Conidiogenesis was studied according to the technique used by Sutton

(1980) i.e. mounting in 3,5% erythrosin in 10% NH_4OH using bright field illumination. Line drawings were done from preparations made in this way. Further elucidation was done by interference contrast and phase contrast lighting using a Leitz SM Lux Pol microscope fitted with a Wild MPS 45 camera. A Wild M 5 A microscope using the same camera, was used for the stereoscopic studies. Better slides were sealed with melted wax for storage of up to 4 days.

Paraffin wax sections of 10 µm thickness were stained with Gurr's Fast Green while freeze sections of 20 - 30 µm thickness were made using a Leitz Kryomat 1700 with a 1310K stage for the study of the evolving locules. All these sections were also mounted in the alkaline erythrosin mentioned above.

TRANSMISSION ELECTRON MICROSCOPY

Strips of agar (1x1x2 mm) containing conidiomata were cut from cultures and immediately fixed in 2,5% glutaraldehyde in either 0,1M phosphate or 0,1 M Na-cacodylate buffer, pH 7,5 containing 0,25% Alcian Blue. Fixation was done under intermittent vacuum for 30 min, thereafter the fixation was continued for another hour at 50° C. Three buffer rinses were followed by post fixation in aqueous OsO_4 for 1 h. Dehydration in acetone was followed by embedding in Epon. The material in Epon was also subjected to intermittent vacuum to facilitate impregnation.

Silver interference colour sections were cut with a diamond knife and contrasted with uranyl acetate and lead citrate. Electron micrographs were taken at 60 KV using a Philips 301 EM.

HISTOCHEMICAL STAINS FOR THE CONIDIAL APPENDAGES

Sudan III stain for fats

0,5% Sudan III in 70% ethanol for 20 min.

Rinse quickly with 50% ethanol. Fats colour red.

Sudan Black B for lipids

Saturated solution in 70% ethanol, stain for 20 min.

Rinse quickly with 50% ethanol. Fats colour a deep blue-black.

Coomassie Brilliant Blue for Protein

Coomassie Brilliant Blue in 7% acetic acid turns protein blue. Fungal material was teased out on a microscope slide and the above-mentioned stain added. The cover slip was put in place and the protein present in tissue would stain blue.

Modified Leifson's Stain (Punithalingam & Woodhams, 1984) used to determine morphology of appendages.

Pelikan Fount India drawing ink for fountain pens, Black, to determine the morphology of appendages.

RESULTS

UROHENDERSONIA PLATENSIS

LIGHT MICROSCOPY

The pycnidium was formed by the enlargement of a hypha at a certain point (Fig. 3 a). Additional hyphae crossing this point became enlarged (Fig. 3 b) and anastomoses formed between the hyphae at the predetermined point of development of the pycnidium (Fig. 3 c). The concentration of hyphae at this point continued to give rise to a loose structure (Fig. 3 d) which became tightly woven (Fig. 3 e). Isodiametric cells developed (Fig. 3 f) and the developing pycnidium became more obviously globose (Fig. 3 g). Additional hyphae crossed the pycnidium and became incorporated into the outer surface thus enlarging the structure (Fig. 3 h). The young pycnidium was at this stage a spherical mass of pseudoparenchyma from which thin hyphae radiated (Fig. 3 i).

This process was described as symphogenous development by Kempton (1919). Internally the centre consisted of large hyaline thin-walled pseudoparenchyma surrounded by smaller more regular, slightly thicker-walled hyaline cells and juvenile plectenchyma (Fig. 2 c). The very prominent layer of darker cells was possibly due to lipids which were also found as encrustations on the vegetative hyphae and crystals (Fig. 5 h) in culture. The particles were stained dark blue with Sudan Black B and no staining was found in material extracted with petroleum ether or chloroform.

Following disintegration of the central hyaline pseudoparenchyma, the

conidiogenous cells became active, a process followed soon after by the formation of the ostiolum. The ampulliform conidiogenous cell (Fig. 2 b) produced the first conidium holoblastically (Fig. 4 b), which was released schizolytically (Hughes, 1971 a). The apical appendage was formed at the same time and grew at a faster rate than the conidium (Fig. 4 d, f, g). The conidial body enlarged and the median septum was formed (Fig. 4 i). The neck of the conidium did not stretch or elongate (Fig. 4 j). However, with production of the second conidium an intercalary cell was formed in the elongated neck of the conidiogenous cell to allow rhexolytic secession (Hughes, 1971b) (Fig. 4 h). The conidiogenous cell now exhibited a frill at its apex where the conidium had been released (Fig. 4 j). The wall of the conidiogenous cell maintained the same diameter throughout (Fig. 4 b, c, j). At this stage, using light microscopy, two horn-like attachments could be seen at the base of the conidium which corresponded to the ring left by the tearing of the basal cell, similar to the frill or pedicel of Pithomyces. These horn-like points were evident in the elongated mucilagenous sheaths which developed later (Fig. 2 d). These frilled conidiogenous cells produced another conidium which was released schizolytically (Fig. 30). Soon after release from the conidiogenous cell, the conidium developed two separate external sheaths, enveloping the conidium (Fig. 4 d). One was formed next to the conidium and frequently displayed a series of irregular ridges, while the basal part formed an extension protruding

from the outer sheath (Fig. 2 d, 5 f). The outer sheath was smooth and basally shorter than the inner sheath. At the apex where the gelatinous tail-like appendage was embedded in a hollow in the conidium, another veil-like appendage which inverted from the body of the conidium, surrounded the tail-like appendage (Fig. 5 b,c,d). This veil-like appendage was present while the conidium was still attached to the conidiogenous cell (Fig. 5 a) and was best demonstrated when mounted in ink (Fig. 6 q, r). Histochemical staining of the appendages gave a positive reaction with Coomassie Blue showing them to be mainly protein. Shortly before release, two additional septa were formed, one below and one above the first. When the full complement of conidia had been produced by a pycnidium, microconidia, which were hyaline, elliptical and aspherical, were produced from small conidiogenous cells (Fig. 4 k, 6 s, t). These microconidia were observed in most cultures grown on richer media (PDA, PCA & MEA) where the pycnidia could remain active for longer periods. The microconidia were enveloped by oversized mucilagenous sheaths which were eventually absorbed. The microconidia might function as spermatia rather than microconidia. The macroconidia became slightly brown in colour after septation was completed (Fig.4 l) and lost their apical appendages at the time of release from the pycnidium.

In cultures grown on various media, especially PCB, the pycnidia elongated considerably while the basal part of the venter became sterile (Fig. 2 a, 5 i). Conidiogenous cells underwent lysis as

soon as they had formed three conidia. Multi-ostiolate pycnidia were only rarely encountered, and only in isolate PREM 47584. Abnormal conidia were extremely rare as only one five-septate conidium was seen during the entire study. Chlamydospores were formed on PCA after six weeks (Fig. 5 J).

The conidiogenous locule became larger as the conidiogenesis proceeded which points to a process of replacement of pseudoparenchyma by meristematic cells.

TRANSMISSION ELECTRON MICROSCOPY

TEM studies on the ultrastructure of U. platensis confirmed that the cell wall of the conidiogenous cell consisted of one layer only (Fig. 7 B). On the outside of this an uneven layer of osmiophilic material was deposited. Some conidiogenous cells had two necks (Fig. 7 B), a condition which could not be seen by means of light microscopy. Each conidium was enveloped in a thick layer of mucus (Fig. 11 B). The conidia were produced holoblastically (Fig. 9 A) and the first conidium was apparently released by schizolytic secession (Fig. 9 A). The second conidium left a delicate frill, derived from the cell wall material of the intercalary cell, above the level of rhexolytic secession (Fig. 9 B). A third and last conidium was produced in a similar manner to the first and apparently released by schizolytic secession as no conidiogenous cell with a second rhexolytic

third conidium was ever seen. After this secession the conidiogenous cell seemed to dissolve and its place was taken by another cell derived from the underlying pseudoparenchyma (Fig. 8 A, 9 A). Elongated conidiogenous cells were occasionally seen in material prepared for TEM (Fig. 7 B, 9 B) but were never observed in fresh material. The osmiophilic material which was present between adjacent cells in the single layered pycnidial cell walls probably consisted of lipids exuded by the peripheral cells, and was thus not a true cell wall layer (Fig. 8 A). The apical appendage consisted of the same electron translucent material as was present in the young conidial cell wall (Fig. 11 A), with an osmiophilic layer deposited on the outside.

DEVELOPMENT OF CELL WALLS OF THE CONIDIUM

The cell wall of the conidium of Urohendersonia platensis consisted of a reasonably electron translucent layer which was externally encrusted with an osmiophilic substance which might have been the same as that found so abundantly in the outer layers of the pycnidial wall (Fig. 8 A). The inner part of this conidial wall was impregnated with a granular substance, giving the impression of the presence of a distinct wall layer (Fig. 10 B). This granular layer was present in mature cell walls only (Fig. 10 C, D). In younger walls, such as

septa (Fig. 10 A), this layer could be observed in the more mature parts of the cell wall only (Fig. 10 B). It would therefore seem that initially the septum consisted of electron translucent material which was impregnated at a later stage of development by granular osmiophilic material which was not deposited in the septal pore (Fig. 10 D).

ULTRASTRUCTURE OF CONIDIA OF OLDER PYCNIDIA OF U. platensis

Conidia retained within an old non-functional pycnidium of U. platensis were still relatively osmiophilic (Fig. 8 A) while extruded conidia were much less so. Eventually some of the cells in the conidia died and appeared to be without cytoplasm (Fig. 12 A, B).

Conidia in the pycnidium (Fig. 8 A), as well as those on the outside (Fig. 12 B), could germinate and anastomoses were formed between adjacent conidial cells (Fig. 12 A). These anastomoses and the presence of mucus (Fig. 13 A) caused the conidial stream to remain intact. It was noteworthy that each individual cell of a conidium could germinate. Germination could even take place into dead conidial cells. These dead conidial cells were also seen by means of light microscopy (Fig. 4 k) where the presence of microconidia indicated the advanced stage of development of the pycnidium. It was noted that conidia could break up into conidial fragments in which some would stain and

others would not. Erythrosin stains cytoplasm only and those fragments which were dead did not stain. The presence of dead cells was confirmed in the TEM study.

MUCILAGE DEVELOPMENT ON THE CONIDIUM OF *U. platensis*

At the stage when mucilage formation took place, the outer osmiophilic layer of the conidium seemed to disintegrate and to release the underlying electron translucent material in the form of mucus (Fig. 11 C). This mucus was most probably analogous to the mucus sheaths seen in the light microscopic studies (Fig. 3 d). After the formation of this mucus only two layers constituted the cell wall; an inner electron translucent layer and an outer osmiophilic layer (Fig. 13 A). When the conidium was fragmented into individual cells, each break took place in the osmiophilic layer of the septum. The osmiophilic layer remained as part of the cell wall of one of the released cells (Fig. 13 B, C) and left small remnants of osmiophilic material behind in the form of minute collarettes on both cells, reminiscent of the frill found on the conidiogenous cell after rhexolytic secession had taken place (Fig. 13 C). A germination tube might be formed on any side of these cells (Fig. 12 A, B).

DEVELOPMENT OF THE APICAL APPENDAGE OF *U. platensis*

The cell wall of the conidiogenous cell and the developing conidium was a continuous homogeneous layer which proliferated apically to form

the incipient appendage (Fig. 11 A). An osmiophilic layer was deposited on the outside of the electron translucent layer. During the development of the conidial cell wall a second osmiophilic layer is deposited as has been described earlier. This inner layer could be seen as a ' rod ' in the basal part of the appendage (Fig. 11 B). Mucilage was formed from the outer layers of the conidial wall by disintegration of the outer osmiophilic and electron translucent layers (Fig. 11 C). If, as seemed likely these layers were analogous to the material of the whole apical appendage, all these structures would be released simultaneously.

TIAROSPORELLA GRAMINIS VAR KAROO

LIGHT MICROSCOPY

The conidioma, a pycnidium, was formed from a single hypha in a meristogenous process (Kempton, 1919). A single hypha enlarged at a predetermined point to form a pycnidium, with a pronounced neck or rostrum, in which locules of conidiogenous cells developed in the hyaline thin-walled pseudoparenchyma (Fig. 14 a). This pseudoparenchyma (Fig. 14 b) was eventually replaced to produce one locule in the elongated pycnidium (Fig. 14 c) which was lined by conidiogenous cells. The pycnidium could grow to a length of 5 mm in artificial culture. The outside was covered in multi-septate hairs (Fig.2 e). Aggregates of pycnidia were formed in culture in which the bases of the individual pycnidia were confluent (Fig. 2 f). In cultures on 2 % MEA three different types of conidiogenous apparatus i.e. conidiogenous cells and conidiophores, could be distinguished (Fig. 16 a -k):

1) branched and borne on conidiophores (Fig. 15 d, e, f);
2) more than one conidiogenous cell on a single conidiophore (Fig. 15 c) and
3) single lageniform conidiogenous cells which could be straight or curved (Fig. 14 a) borne on a conidiophore.

All these conidiogenous cells produced identical conidia by the holoblastic process (Fig. 15 g). No evidence was found of any thicke-

nings or annellations.

The conidiogenous cell layer was covered by mucilaginous material probably exuded by the conidiogenous cells (Fig. 14 f, g, 15 a). This interfered with the staining procedure. The development of the apical appendage of the conidium is illustrated in Fig. 14(f). The apical appendage was produced simultaneously with the conidium as an outer cell covering although it was loose at the base. As the conidium was released, this mucilaginous layer everted over the apical point of the conidium (Fig. 14 e), persisted and remained intact (Fig. 15 h, i, j, k, l). The appendage stained well in modified Leifson's stain (Punithalingam & Woodhams, 1984) (Fig. 15 h, i, j) where it showed up as an envelope the size of the conidium. The mature conidium was markedly guttulate (Fig. 15 k).

There was marked mucus formation in young active pycnidia where the process of conidiogenesis had just begun (Fig.15 a). The origin of the mucus was believed to be the result of the replacement of the pseudoparenchyma by the conidiogenous cells, analogous to what had been reported for Bartalinia robillardoides in this study. This mucus and the apical appendage were removed when the conidiomata were extracted with glacial acetic acid under vacuum and heated to 70°C. The composition of this appendage and the mucus was not determined but was assumed to consist of the remnants of the cell walls of the replaced cells.

TRANSMISSION ELECTRON MICROSCOPY

Cell walls of the pycnidial plectenchyma of Tiarosporella graminis var karoo consisted of homogeneous electron translucent material (Fig. 17 A). Exudates occurred between adjacent cells which gave the impression of the presence of more than one layer, especially in the case of the peripheral cells (Fig. 17 A). The exuded osmiophilic outer layer could possibly consist of lipids secreted by cells of the outer cell layers. The inner cell layers of the pycnidial wall consisted of thick-walled cells, which had a particularly angular appearance (Fig. 17 A), and each contained a large number of mucus vesicles (Fig.19 B).

Apparently, the conidiogenous layer was derived from the cells of the pycnidial wall by conversion (Fig. 18 C). After the conidia had been released, the cells of the conidiogenous apparatus underwent lysis and the resultant mucilaginous material remained in the conidiogenous locule (Fig. 17 C). Occasionally more than one conidiogenous cell might develop from each conidiophore (Fig.17 B). Conidia arose holoblastically from the conidiogenous cells (Fig. 18 A). Electron micrographs illustrated that the appendage was present much earlier (Fig. 18 B) than could be detected by light microscopy and that it consisted of a substance different from that of a mucilaginous material exuded by the adjacent cells (Fig.17 C, 18 A,B). The incipient appendage extended over the entire developing conidium, enveloping it from the conidiogenous locus upwards (Fig. 18 B). This appendage

was visible as a sheath with a texture differing from that of the surrounding mucus. The mucus was discernible in electron micrographs due to the use of Alcian Blue during the initial fixation procedure. Different chemical constituents in the mucilages of the appendages and the surrounding lytically derived mucus may have given rise to this effect (Fig. 17 C). Conidia were released by schizolytic secession (Fig. 18 A) and conidiogenesis was apparently monoblastic.

The morphology of the conidiogenous cell confirmed that one continuous cell wall layer was present on both the conidiogenous cell and the conidium (Fig. 18 B). No changes in wall structure occurred at the neck of the conidiogenous cell, thus confirming the light microscopic observations. The conidial cell wall, however, consisted of two distinct layers, the inner electron translucent layer similar to that of the conidiogenous cell and an outer osmiophilic layer (Fig. 19 A). The guttulate appearance of the conidium was due to electron translucent material which formed globular structures (Fig. 19 A).

BARTALINIA ROBILLARDOIDES

LIGHT MICROSCOPY

Formation of conidiomata was symphogenous (Kempton, 1919). A hypha enlarged (Fig.20 a) and normal sized hyphae crossing this point concentrated (Fig. 20 b) to enlarge and eventually form a loosely arranged network which formed a very loosely woven pseudoparenchyma (Fig. 20 c). This process was spread over a large area to form series of conidiomata. These structures were multilocular (Fig. 2 h) in the initial stages and could open to the outer surface through an ostiolar canal formed in the dome (Dodge, 1930) or vault of the conidioma. Multilocular conidiomata were formed on CLA, PCA, MEA and carnation leaflets and were considerably larger than the pycnidia described for this fungus (Sutton, 1980). Within these locules conidiogenous cells were produced by lysis of the innermost hyaline pseudoparenchymatous cells (Fig.20 d). The locules were separated by columnar pseudoparenchyma, where cells were closely packed and oblong with few intercellular spaces (Fig. 20 e, f, h) and which formed columns apparently supporting the vault of the conidioma. The columns separating the locules were broken down by transformation of the pseudoparenchyma into conidiogenous cells (Fig. 20 h). The elongated cells were apparently hyphae from which the conidiogenous cells arose (Fig. 20 e, l) and formed hyphal strands bearing the conidiogenous apparatus The locules were progressively enlarged by replacement of the pseudo-

parenchyma by conidiogenous cells (Fig. 20 g). Strands of mucus flowing from the basal layers were observed between the conidiogenous cells. These could be the result of lysis of the hyaline thin-walled pseudoparenchyma. This mucilaginous layer formed an impermeable layer (Fig. 20 i, 21 a) which made fixation of the material for EM studies very difficult and also interfered with the uptake by the conidiogenous cells of all stains except alkaline erythrosin.

The conidiogenous apparatus consisted of conidiophores (Morgan-Jones et al., 1972, Fig. 4) which supported branched and unbranched conidiogenous cells (Fig. 21 c, b). Two morphologically distinct types of conidiogenous cells were observed viz. filiform and ampulliform (Fig. 20 e). Both produced morphologically similar conidia. The morphology of the conidia agreed with those described by Morgan-Jones et al. (1972) and Sutton (1980). The conidia were produced holoblastically as an elongation of the conidiogenous cell (Fig. 21 a). The transverse septum separating the conidium from the conidiogenous cell was formed very early followed by or simultaneously with development of the apical appendage (Fig. 21 b). The apical cell lost its cytoplasm when the appendage was fully grown (Fig. 20 i, j). The median septum of the

developing conidium was formed by microcephalic septation (Hawksworth, Sutton & Ainsworth, 1983). Additional growth took place (Fig. 21 h) during which a total of five septa were formed. As the conidium was released from the conidiogenous cell a single basal off-centre appendage appeared, which was formed from the cell wall of the basal cell. The apical appendage diverged and formed two to four, usually three arms (Fig. 20 i, k).

Additional conidia were formed by rhexolytic secession, similar to what was found in U. platensis (Fig. 21 b, f, g) where the intercalary cell could be seen. No alterations to the neck of the conidiogenous cell could be detected by means of light microscopy. The additional cell responsible for the rhexolytic secession could attain a length equal to the filiform conidiogenous cell itself (Fig. 21 f). In older conidiomata, what appeared to Morgan-Jones et al. (1972) to be annellations, were detected on the necks of the conidiogenous cells (Fig.26 n). However, these necks were wide with no differentiation in staining as would be expected with annellides. No periclinal thickenings, lengthening or shortening of the conidiogenous cells were observed.

The morphology of the conidioma of B. robillardoides was studied in detail because it was different from that which had been described for

the genus. The conidioma proved to be a eustromatic, multilocular fructification instead of a unilocular pycnidium.

Serial sections are depicted in Fig. 22 and 23 to illustrate the construction of the conidioma as seen on horizontal and vertical planes. It is interesting to note that only one ostiole for each conidioma was seen in all the sections that were made. This was unexpected as folds on the top of the conidioma (Fig.24 b, c) had given the impression that more than one ostiole, and perhaps even clypeate openings, could be present. The ostiole could be seen in the horizontal plane (Fig. 23 a - j) and in the vertical plane (Fig. a - j). These figures illustrate the convoluted nature of the locules formed by the replacement of pseudoparenchyma by the conidiogenous cells, which thus formed the enlarged locules.

After studying these sections it was possible to suggest a three-dimensional picture of the development of the conidioma (Fig. 25 a, b, c). The ontogeny of the conidioma was described earlier. Conidiogenesis started from several loci simultaneously to form conidiogenous locules which became enlarged and ultimately confluent with one another. Finally there were only a few divisions left which would presumably also be converted into conidiogenous cells since they consisted of pseudoparenchyma.

TRANSMISSION ELECTRON MICROSCOPY

The conidiomal plectenchyma consisted of isodiametric cells with a cell wall structure similar to that of the inner pseudoparenchyma from which the conidiogenous apparatus was derived. The cells were covered externally with a layer of osmiophilic particles, possibly lipids, which were exuded from the cells and which formed an apparent additional layer between adjacent cells (Fig. 27 A). Osmiophilic substances were, however, also present between the conidiogenous cells and in the underlying cell layers where conversion took place (Fig. 27 B).

Conidiogenous cells were borne on conidiophores, each of which may produce more than one conidiogenous cell (Fig. 27 B). The wall structure of the conidiogenous cells was similar to that of the underlying cells (Fig. 28 A). These cells repeatedly formed a conidiogenous apparatus, each of which produced new conidiogenous cells (Fig. 28 B). The dead conidiogenous cells underwent lysis and the resultant mucus filled the conidiogenous locule. Apparently mucus was also produced by vesicles in the underlying pseudoparenchyma (Fig. 27 A). A frill consisting of virtually only the outer

osmiophilic material was characteristic of the older conidiogenous cells (Fig. 27 A) and only one frill developed on any specific conidiogenous cell (Fig. 27 B).

The mature conidium was circular in transverse section (Fig. 29 A) and eventually contained five live cells, the apical (fifth) cell lost its cytoplasm soon after formation of the extended three-armed appendage (Fig. 28 C). These arms, in which no cell lumen was distinguishable, consisted mainly of electron translucent material arising from the inner layers of the wall of the conidium (Fig. 29 B). Mucilaginous material surrounded the appendages (Fig. 29 A, B). No septal pores were observed in the conidia, even in accurately orientated longitudinal sections (Fig. 29 A).

DISCUSSION

There are indications that the process of conidiogenesis as observed in culture may differ from that seen under natural conditions. Furthermore it appears that the onset and duration of conidiogenesis is dependent on the amount of nutrients available. For example, on poor media such as CLA and water agar, conidiomatal initials appeared on the fourth day after inoculation, were ready for study by the fifth day and had stopped forming conidia by the ninth day. Cessation of conidiogenesis was also observed at a much earlier stage of development of the conidioma on the poor media than was the case on richer media such as PDA. Cultures grown on PCA took up to seven days for the first conidiomata to develop and development of new conidia was observed for a period of up to three weeks with the continued formation of new conidiomata.

Cultures on 2% MEA and PDA took much longer (12 days) to mature. Most conidiomata developed under the surface and showed poor ostiole formation. However, when a single carnation leaflet was put on the medium at the time of inoculation conidiomata were formed much earlier.

In cultures which were grown under conditions where the agar did not desiccate as rapidly, exceptionally long and prominent necks formed on the conidiomata. Sections of older pycnidia of U. platensis

and T. graminis var karoo showed that conidiogenesis was confined to the young apical areas of the neck and that the inferior areas of the pycnidia could be filled with dead hyaline cells.

This investigation showed that a conidiogenous locule developed in the growing pycnidium by lysis of some of the internal pseudoparenchyma. Conidiogenous cells arose from the pseudoparenchyma thus exposed and the conidia, as well as their originating cells, were surrounded by what appeared to be a mucilaginous mass. This possibly consisted of chitin-like or polysaccharide substances and was apparently the end-product of the breakdown of pseudoparenchyma as well as of dying conidiogenous cells.

It became apparent that conidiogenous cells in all three species of fungi examined had a limited life span. Bartalinia and Urohendersonia formed three conidia only per conidiogenous cell which then degenerated. As a conidiogenous cell layer died off, it was replaced by new fertile cells which developed from the previously sterile pseudoparenchyma. This process resulted in the formation of an enlarging locule within the body of the pycnidium as well as in the conidiostroma. This locule was lined with fertile cells and filled with conidia surrounded by the mucilage referred to above. This mucilaginous material interfered with the fixation and resin impregnation for TEM investigation.

In Bartalinia and Tiarosporella, described as pycnidial (Sutton, 1980), the conidiogenous locules developed from more than one locus. The cavities thus formed eventually coalesced to form one large, apparently unilocular, fructification. In Tiarosporella there was no discernible differentiation of the interlocular pseudoparenchyma but in Bartalinia columnar associations of cells formed by hyphal strands bearing conidiogenous cells could be identified between the locules.

The cell wall of the developing conidia in Urohendersonia, Bartalinia and Tiarosporella were shown to arise by extension of the whole thickness of the uniform cell wall of the conidiogenous cell and no apparent annular scar was observed in individual conidiogenous cells. Succeeding conidia did not form bands of circular thickenings of the walls as would be the case in typical annellidic conidiogenesis. The apex of the conidiogenous cell was also virtually as large as the base of the conidium and even though a frill was present in the neck region of the conidiogenous cell of Urohendersonia and Bartalinia, there was no evidence any periclinal thickenings. Furthermore, there was no evidence of an increase in width of the conidiogenous cell at the level of secession or of lengthening or shortening of the cell.

The conidiogenous cells found in Urohendersonia and Bartalinia did not conform to the currently accepted concepts of a phialide (Kendrick & Carmichael, 1973), percurrently proliferating annellides (Morgan-

Jones et al, 1972) or tretic conidial ontogeny (Ellis, 1971) and necessitated the introduction of a modified terminology.

The first conidium was typically holoblastic and seceded by schizolytic division of the separating septum. As, however, the wall of the conidiogenous cell was completely uniform no remnant of an outer layer remained as an annular scar. The second conidium was formed by growth of the separating septum and the conidiogenous cell was not a percurrent annellide. The second conidium was released by rhexolytic secession and left a pronounced cylindrical cell wall, the frill, behind. At this stage there was no evidence of periclinal thickenings of the walls. The third conidium was produced holoblastically from the entire cell wall within the frill left by the rhexolytic secession, which constituted the conidiogenous locus, proliferated. This last conidium seceeded by a schizolytic process as no conidiogenous cells with more than one frill were seen by means of TEM. The conidiogenous cells died and were replaced from the underlying pseudoparenchyma after formation of the third conidium.

A term has to be proposed to describe the process outlined above and the suggested term is 'thysanoblastic' (etym. : 'thysano' (Greek = frill) and 'blastos' (Greek = to sprout)). The process differs from the phialidic ontogeny insofar as no periclinal thickenings are formed, from the annellidic ontogeny as no change in length of the conidiogenous cell takes place and form the tretic ontogeny insofar as all layers of the wall of the conidiogenous cell take part in the formation of the successive conidia (Fig. 30).

Dodge (1930) and Stolk (1963) suggested that the conidiogenous locule developed by the lysis or degeneration of existing hyphae in the central region of a pycnidium or conidiostroma. Di Cosmo & Cole (1980) described central hyphae which seemed to differentiate synchronously into conidiophores at an early stage. During this study, however, the innermost cells of the pseudoparenchyma of the conidioma were seen to enlarge considerably and appeared to form the locule by disintegration.

Vobis and Hawksworth (1981) described various types of pycnidial ontogeny but did not give explicit details of the processes leading to the formation of the conidiogenous locules. Nag Raj (1981) however, made particular mention of schizogenous and lysigenous activity as the most common mechanisms of formation of locules in the coelomycetes. The observation that the conidiogenous cells are active for a very short time only before being replaced and thus forming a conidiogenous locule which continues to enlarge, was significant. It is tempting to speculate on the manner in which these processes could have developed. It is possible that reserves of nutrient materials in each cell of the conidioma are limited and thus allow the formation of only a few conidia. It is also conceivable that the increasing numbers of conidia which have to be accommodated within the locule would exert considerable pressure on the conidiogenous cells which would be unable to survive. Successive regeneration from the cell layers thus exposed could also

account for the development outlined above.

The two considerations of limited nutrients reserves and restricted space do not apply to the Hyphomycetes and are possibly the reason why thysanoblastic ontogeny has not been found in these fungi. The observation that conidiogenous cells in old conidiomata can elongate occasionally (Fig. 24 e) to form an apparent second rhexolytic scar can possibly be proposed as supporting evidence considering that most conidia have at this stage been released from the conidioma. There would thus be sufficient space to allow such additional growth.

It is not surprising to find that the structure of the cell walls of the conidiogenous apparatus was the same as that of the walls of the cells which made up the conidioma. However, the walls of conidia and somatic hyphae were more complex in structure. It is possible that the more complex cell wall found in these structures is an adaptation to exposure to an external environment.

Some morphological characters which have been generally used in the taxonomy of the Coelomycetes, have to be reappraised in the light of the present study. The wall thickness of a pycnidium is accepted by Morgan-Jones & White (1983) as an acceptable species-delimiting criterion. The present study has shown, however, that this is not

a constant characteristic and is dependent on the stage of maturity of the conidioma. Boerema & Loerakker (1985) used differentiation of the pycnidial wall as a species character by distinguishing scleroplectenchyma which stained red with the addition of Lugol's iodine. This situation raises the question of how important to taxonomy the structure of the conidioma really is.

Sutton (1973) stated that no system of classification had been proposed that would accommodate the variability and diversity in form of the coelomycetous conidiomata in a satisfactory manner. Initially a pycnidium was considered a closed asexual fructification in which a defined ostiole was formed (Sutton, 1968). The definition has, however, changed to exclude details of ostioles or the mechanisms of conidial dispersal (Sutton, 1980) but retained the concept that the whole inner surface of the lumen is lined with conidiogenous cells (Hawksworth et al., 1983). It also became clear (Sutton, 1980) that pycnidia and stromata were not the only fructifications in the coelomycetes and that intergradation between the various types was possible. The complexity of this situation has been adequately illustrated in the present work. For example, Bartalinia from a natural substrate was described by Morgan-Jones et al. (1972) as forming unilocular pycnidia (Table 2) and yet consistently formed multilocular stromata on artificial media. A similar situation has been reported for Hyalotiella transvalensis Papendorf which was isolated as stromata

from soil while the other species in the genus formed pycnidia on their natural hosts (Nag Raj, 1981). In the present study, the various locules in Bartalinia coalesced to form a single locule opening through a single ostiole. It is, therefore, not impossible that instead of being a pycnidium the conidioma in this genus may be stromatic and that a similar situation occurs in other genera.

A third criterion that was found to be unstable was the size of the conidia in artificial culture. The conidia of Urohendersonia platensis PREM 47854 on the host and in culture were initially decidedly larger (Fig. 2 e) than those described for the species (Nag Raj & Kendrick, 1971) but after subculturing they became smaller to eventually agree with the dimensions given for the species. On the other hand, the conidia of the two isolates of Bartalinia became larger with repeated subculturing until they exceeded the given dimensions by an average of 10 µm. In addition the appendages were much more variable than had been described and two- and four - armed apical appendages as well as very long basal appendages were found.

Morgan-Jones et al. (1972) described the apical appendages of Bartalinia as having a cell lumen and persistent protoplasmic continuity with the arms of the appendages at maturity. The present study

showed, however, that the arms of the appendages consisted of cell wall material only and that the apical cell from which the appendages arose died as soon as the arms reached full size. Furthermore, there were no differences in structure between the apical and basal appendages (Fig. 29 A, B).

The appendages of U. platensis were described by Nag Raj & Kendrick (1971) as being an extension of the sheath of the conidium. The TEM studies reported here have, however, shown that they consisted of cell wall material as was the case in Bartalinia. Since the cell walls of these two fungi were different in structure, it followed that the appendages were also different. The cell wall of the conidium in Urohendersonia consisted of a single layer of material which became impregnated with osmiophilic material. As the impregnation did not occur in the apical appendage itself, the appendage stayed the same as the immature conidium wall.

This study has illustrated that, when studying an organism in depth, it is essential that every available method of obtaining data be used. Light microscopy on its own is inadequate as are all the other techniques described here when used in isolation.

Study of conidiogenesis of Urohendersonia platensis and Bartalinia robillardoides has shown these species to be closely related and has confirmed the close taxonomic proximity suggested by Sutton (1980).

Light microscopy used on its own can be very misleading as shown

by electron microscopy. TEM of the frill remaining after secession of the second conidium showed that it frayed into microfibrills to present an irregular edge. When seen under the light microscope the frayed edges gave the appearance of multiple annellations. The results presented here are sufficient evidence that great care has to be taken in the presentation of results and interpretations. It is essential that drawings of structures be as accurate and complete as possible and illustrate exactly what was actually seen. Even then errors in interpretations and illustration are possible due to the small size of the cells involved. Using light microscopy the conidiogenous cell of Urohendersonia platensis appeared to have two annellations (Fig. 6 p) which were in fact frayed edges of the single frill described earlier (Fig. 30). The ' drawing convention ' used by Minter, Kirk & Sutton (1982, 1983) would also be misleading for the results reported here, as the wall of the conidiogenous cell was completely uniform with no distinct outer layer and yet it had a defined growth zone which involved the whole of the cell wall of the conidiogenous cell but not the whole of the secession fracture.

This investigation has again emphasized the fundamental question of the validity and stability of the currently accepted morphological taxonomic characters. The morphology and ontogeny of the conidiomata were shown to be substrate-dependent, conidial dimensions were shown to vary and light microscopic evaluation of conidiogenesis was unreliable.

These commonly used criteria were thus unreliable and it is again apparent that, in attempting to identify and classify members of the Coelomycetes, a wide range of characteristics, obtained from a broad technological base, must be considered. This study has shown that accepted custom frequently requires re-appraisal and that new delimiting criteria perhaps need to be identified. What these criteria will be only time will tell.

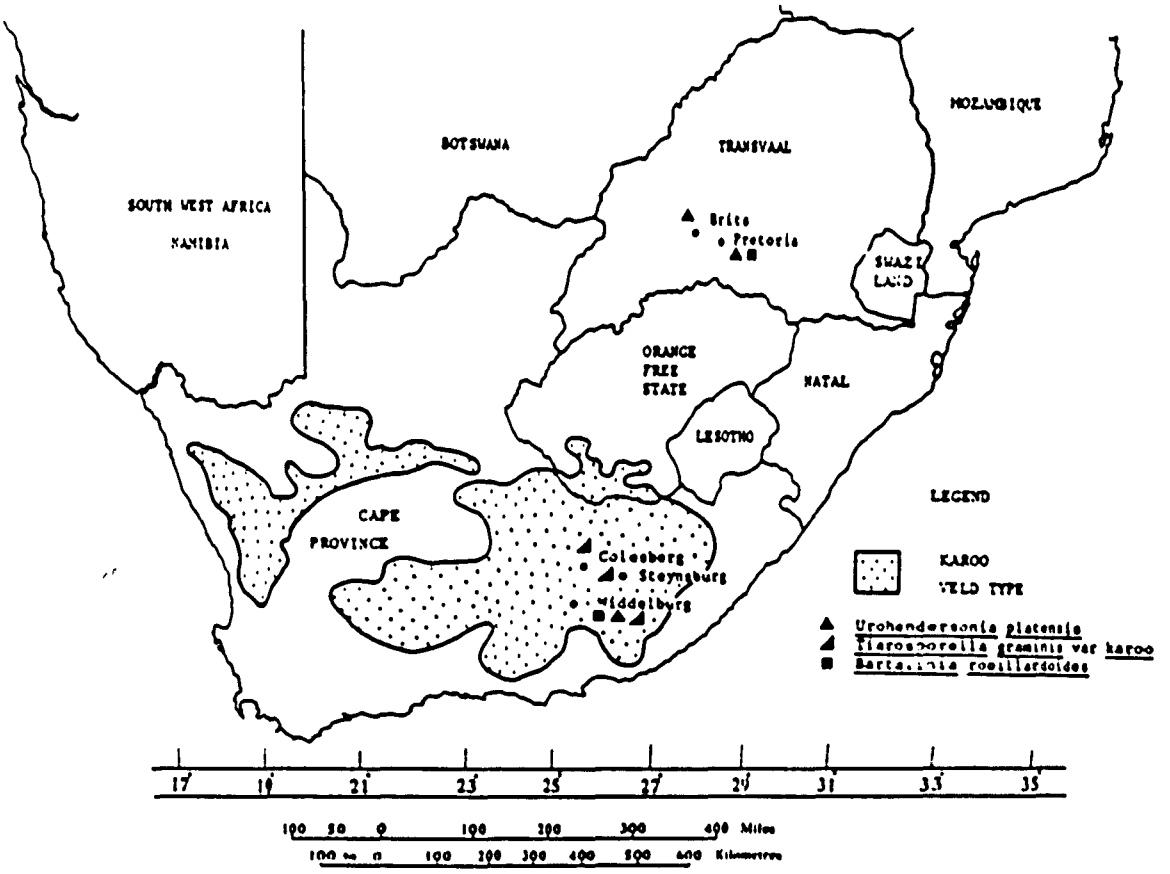


Fig. 1. Distribution map of the three coelomycetes

Fig. 2 The three Coelomycetes

Urohendersonia platensis

a Pycnidia with elongated necks grown on potato carrot broth

b Conidia showing the apical appendage attached to the ampulliform conidiogenous cells (Bar = 10 µm)

c Vertical section through a pycnidium showing the conidiogenous layer with conidiogenous cells and conidia (Bar = 25 µm)

d Mature conidia from an initial culture of PREM 47854 which were unusually large, showing the three mucilaginous sheaths and apical appendage (Bar = 10 µm)

Tiarosporella graminis var karoo

e Elongated necks of pycnidia covered with long hairs, developing from an aggregate of pycnidia (Bar = 1 mm)

f Vertical section of an aggregate of pycnidia where the confluence of the pycnidia is shown (Bar = 50 µm)

g Conidium with veil-like appendage, stained with erythrosin (Bar = 10 µm)

Bartalinia robillardoides

h Multilocular conidioma grown on agar (Bar = 100 µm)

i Conidiogenous cells, one bearing a conidium and a mature conidium showing two of the three apical appendages and the off-centre basal appendage as well as three septa (Bar = 10 µm)


a
b
c
d
e
f
g
h
i

Fig. 3 Ontogeny of the pycnidium of Urohendersonia platensis

a Hyphae enlarging where they cross (Bar = 50 µm)

b More hyphae concentrating at the point of enlargement (Bar = 50 µm)

c Anastomoses between enlarged hyphae (Bar = 10 µm)

d Concentration of hyphae at point of enlarged hyphae (Bar = 50 µm)

e Isodiametric cells forming where hyphae cross (Bar = 50 µm).

f Same as ' e ' , but focussed on upper surface (Bar = 50 µm)

g Young pycnidium now discernable as a round ball of pseudoparenchyma

h Enlarged hyphae crossing the young pycnidium on the outside

i Established developing pycnidium

g - i (Bar = 50 µm)

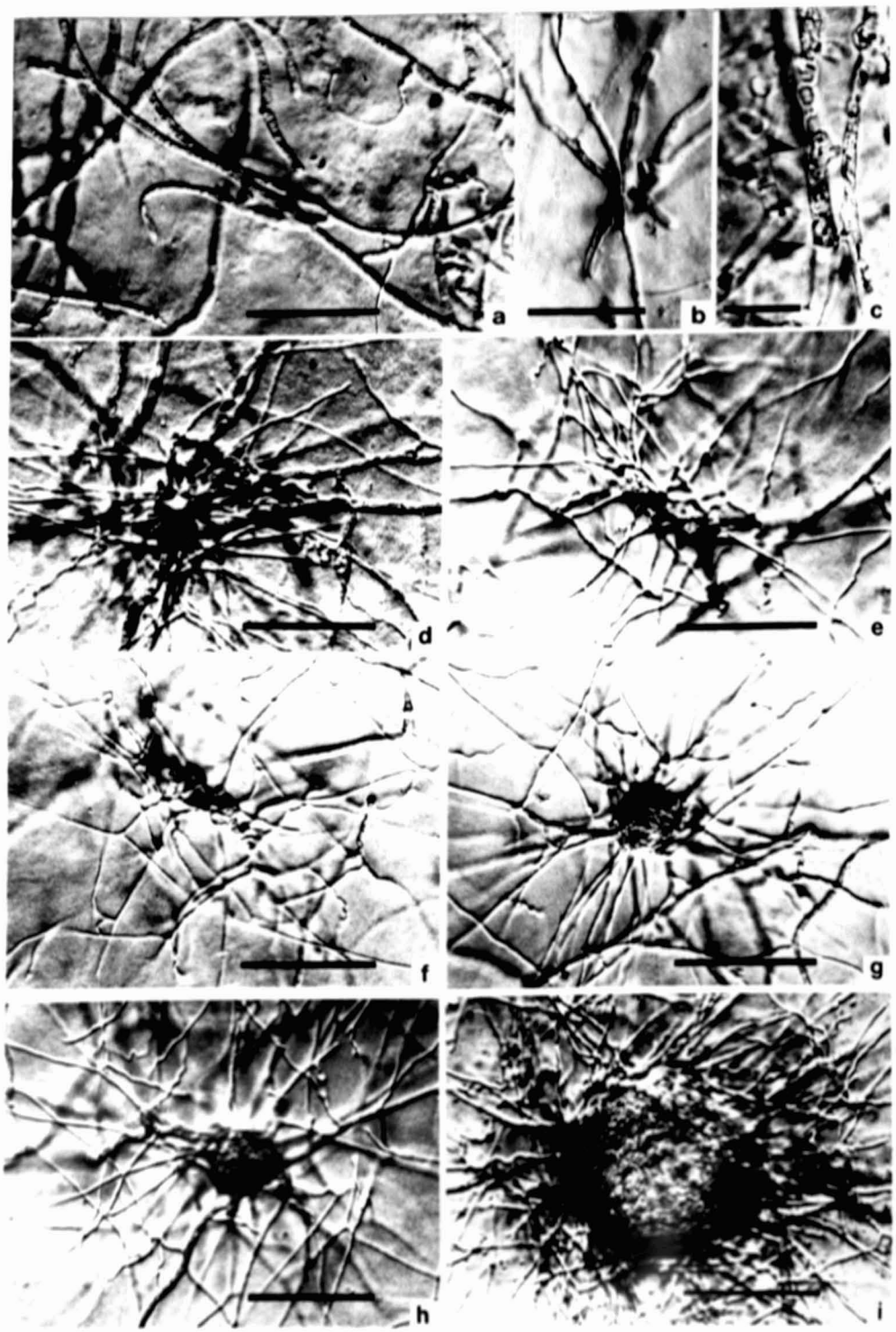

a
b
c
d
e
f
g
h
i

Fig. 4 Conidiogenesis in Urohendersonia platensis

a Conidiogenous cells with raised necks and the first indications of bud formation

b Conidiogenous cells with cell walls of even thickness

c Conidiogenous cell with an elongated neck, showing what appears to be formation of the basal septum

d Conidiogenous cell where development of the apical appendage has just begun

e A conidiogenous cell with a more prominent ' bud '

f Conidiogenous cell with further development of the conidium and apical appendage

g Further stage in the the development of the conidium and appendage, which by this time is already curved, typical of the species

h Intercalary cell between conidium and conidiogenous cell

i Conidium showing the development of the outer gelatinous sheaths. The primary septum is almost fully developed

j Indications of the very delicate frill can be seen on the conidiogenous cell

k Microconidia, held together by their mucilaginous sheaths, and an adjacent conidium, where the additional septa are forming

l A mature conidium where septation is completed and the mucilaginous material surrounding the conidium is evident

(All bars = 10 µm).

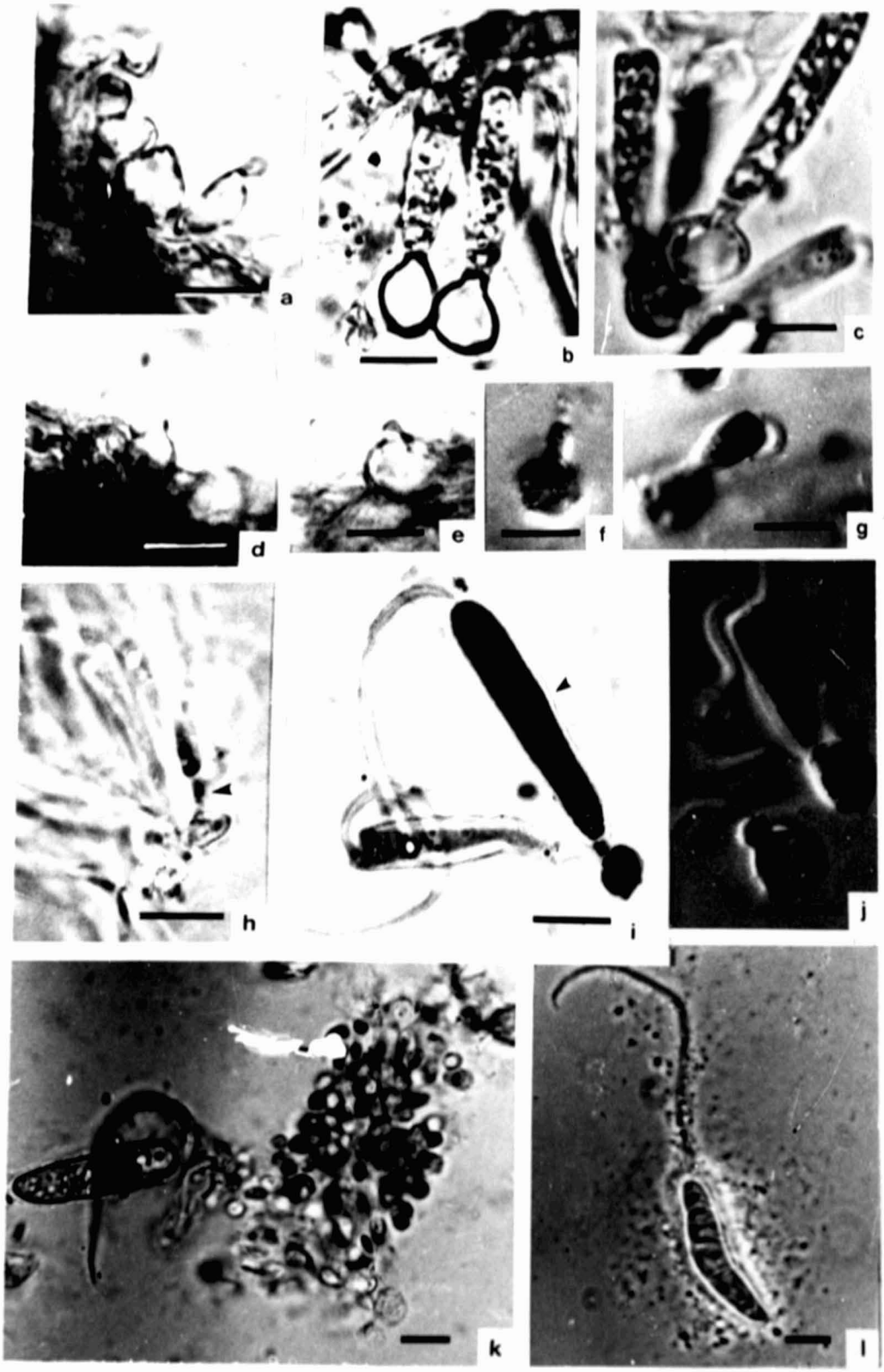

a
b
c
d
e
f
g
h
i
j
k
l

Fig. 5 Development of the mucilaginous appendages and other features of Urohendersonia platensis

a Conidia attached to conidiogenous cells in the pycnidium. The apical mucilaginous sheath has inverted over the apical part of the conidium

b A released conidium where the sheath can be seen as a veil over the apical appendage

c, d & e
Views of the mucilaginous shaeth which inverts over the apical part of the released conidium

f Inner sheath at the basal part of the conidium

g Mature conidium with typical appendage bent back towards the body of the conidium, which is constricted at the septa

h Crystals which form in the medium

i Liquid culture of U. platensis in which the long neck of the submerged pycnidia can be seen

j Darkened intercalary chlamydospores formed on PCA

(Bar = 10 µm) except i

Fig. 5 Development of the mucilaginous appendages and other features of Urohendersonia platensis

a Conidia attached to conidiogenous cells in the pycnidium. The apical mucilaginous sheath has inverted over the apical part of the conidium

b A released conidium where the sheath can be seen as a veil over the apical appendage

c, d & e
Views of the mucilaginous shaeth which inverts over the apical part of the released conidium

f Inner sheath at the basal part of the conidium

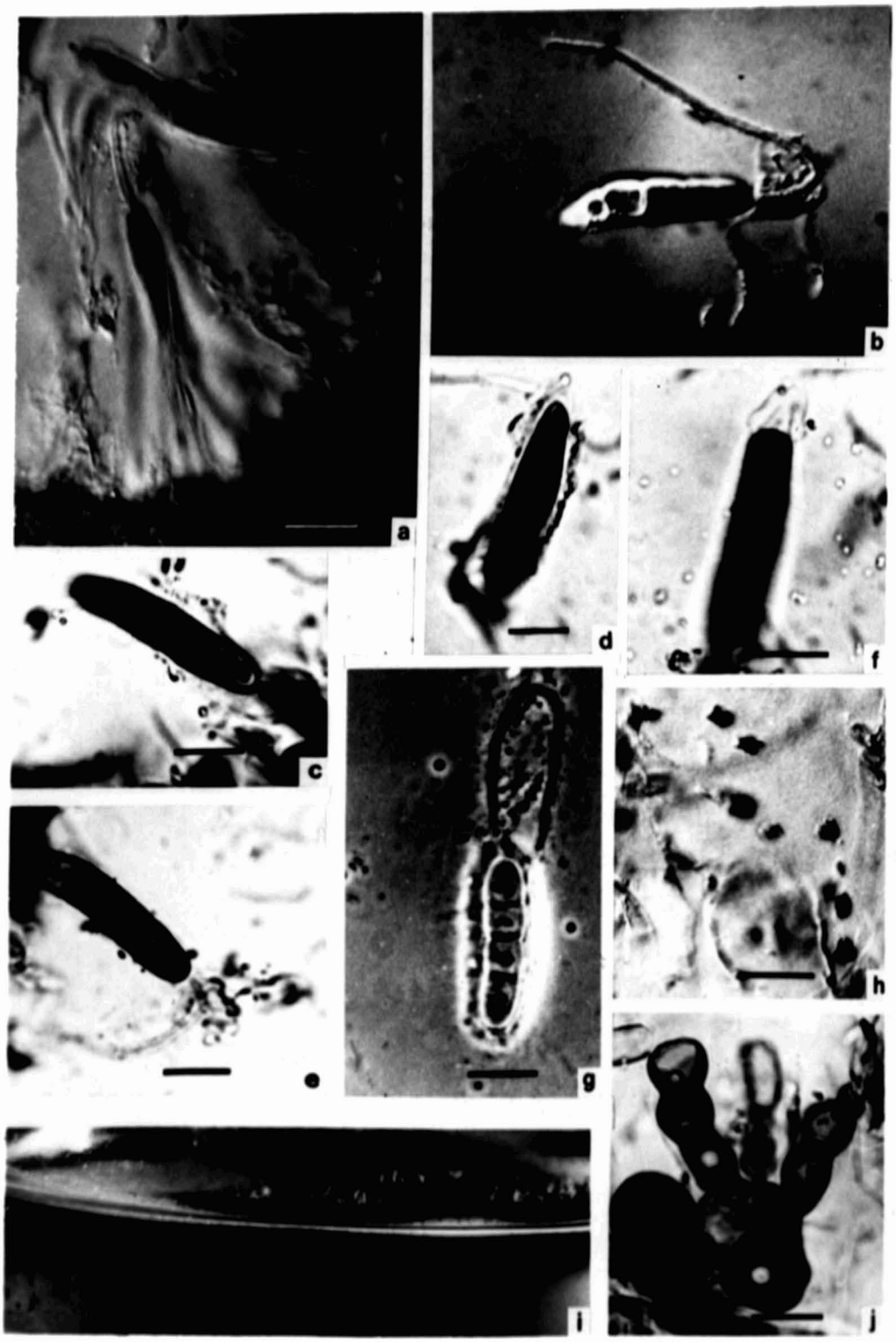
g Mature conidium with typical appendage bent back towards the body of the conidium, which is constricted at the septa

h Crystals which form in the medium

i Liquid culture of U. platensis in which the long neck of the submerged pycnidia can be seen

j Darkened intercalary chlamydospores formed on PCA

(Bar = 10 µm) except i


a
b
c
d
e
f
g
h
i
j

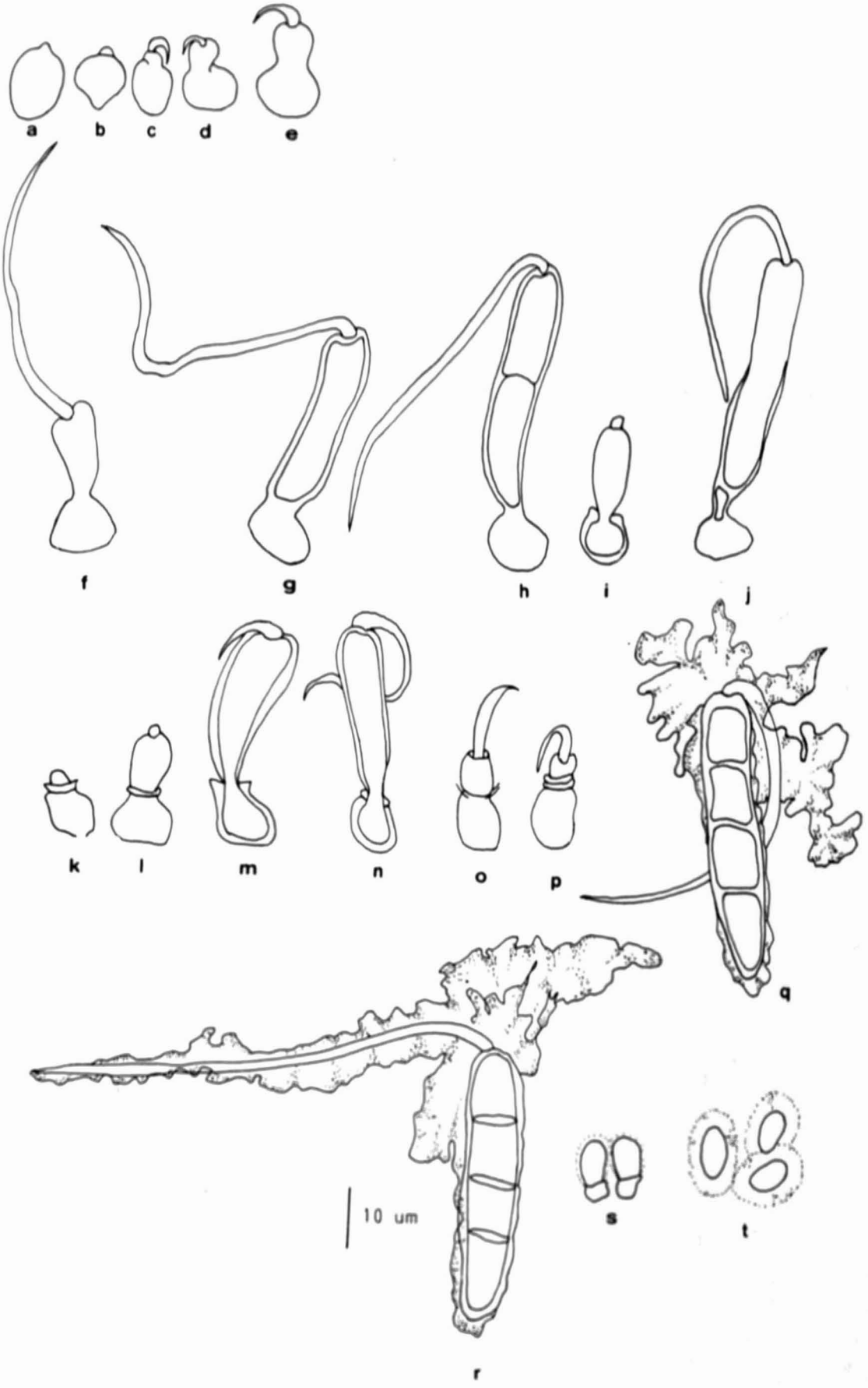

a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
s
t
10 um

Fig. 7 Electron micrographs of Urohendersonia platensis

A Conidiogenous cell showing developing conidium and uniform cell wall structure, similar to that of the underlying pseudoparenchyma (Bar = 1 µm)

B Conidiogenous cell (CC) with two necks and elongated body (Bar = 1 µm)


A
CC
B

Fig. 8 Electron micrographs of Urohendersonia platensis

A Section through the pycnidial wall showing the plectenchyma (PL) peripherally, the pseudoparenchyma (PP) and the conidiogenous cell (CC) bearing a conidium (C) as well as a dead conidiogenous cell (DC). (Bar = 10 µm)

B Section through an old pycnidial locule showing the dead pseudoparenchyma (PP) and two germinating conidia. The locule is filled with mucilage. (Bar = 1 µm).


C
DC
CC
PP
PL
A

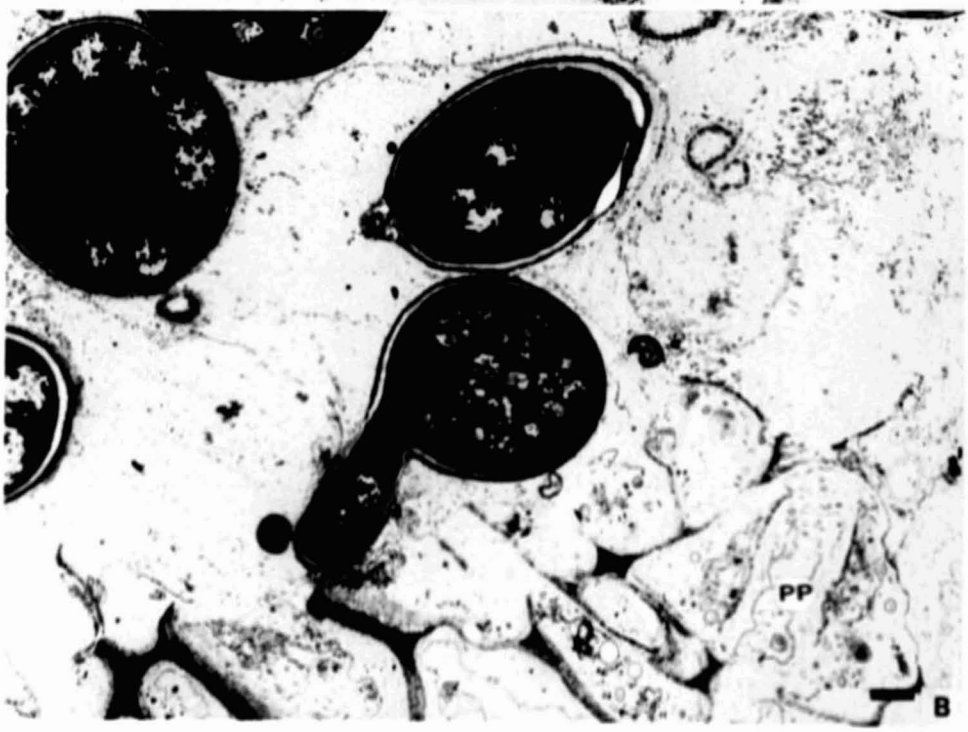

PP
B

Fig. 9 Electron micrographs of Urohendersonia platensis

A Dead conidiogenous cells (DC) are being replaced by conidiogenous cells from the pseudoparenchyma (PP). The conidiogenous locus (CL) and apical appendage (AA) are shown on a young conidiogenous cell, as well as an older conidiogenous cell where the intercalary cell (IC) supported the conidium (C) before rhexolytic secession would take place (Bar = 1 um).

B Conidiogenous cell (CC) bearing a conidium (C) with an apical appendage (AA) showing the frill left by the rhexolytic secession (arrowed) (Bar = 1 µm)


C
M
IC
AA
DC
CC
CL
DC
PP
A
AA
C
CC
B

Fig. 10 Electron micrographs of cell wall formation in the conidium of Urohendersonia platensis.

A The first stage of septum formation showing the formation of the electron translucent layer being impregnated with osmiophilic material (Bar = 1 µm)

B The second stage where the median section of the septum stays electron translucent while impregnation of the layer proceeds in other parts of the cell wall (Bar = 1 µm)

C Further development of the cell wall showing accumulation of osmiophilic material in the electron translucent layer while the outer osmiophilic layer starts to darken (Bar = 1 µm)

D The completed septum where the septal pore is shown as a electron translucent layer with Woronin bodies on either side (Bar = 1 µm).


A
B
C
D

Fig. 11 Electron micrographs of the apical appendage of *Urohendersonia platensis*

A The young conidium showing the uniform cell wall which consists of electron translucent (ET) and osmiophilic material (O) which extends into the apical appendage (Bar = 1 µm)

B The older conidium where the mature cell wall consisting of two osmiophilic layers (O) and two electron translucent layers (ET) is shown. In the base of the apical appendage the osmiophilic material forms a 'rod' which does not extend into the appendage. Mucilage surrounds the conidium. (Bar = 1 µm)

C The older conidium showing the disintegration of the outer osmiophilic layer (O) and the underlying electron translucent layer (ET) which are both apparently released in the form of mucilage (Bar = 1 µm)

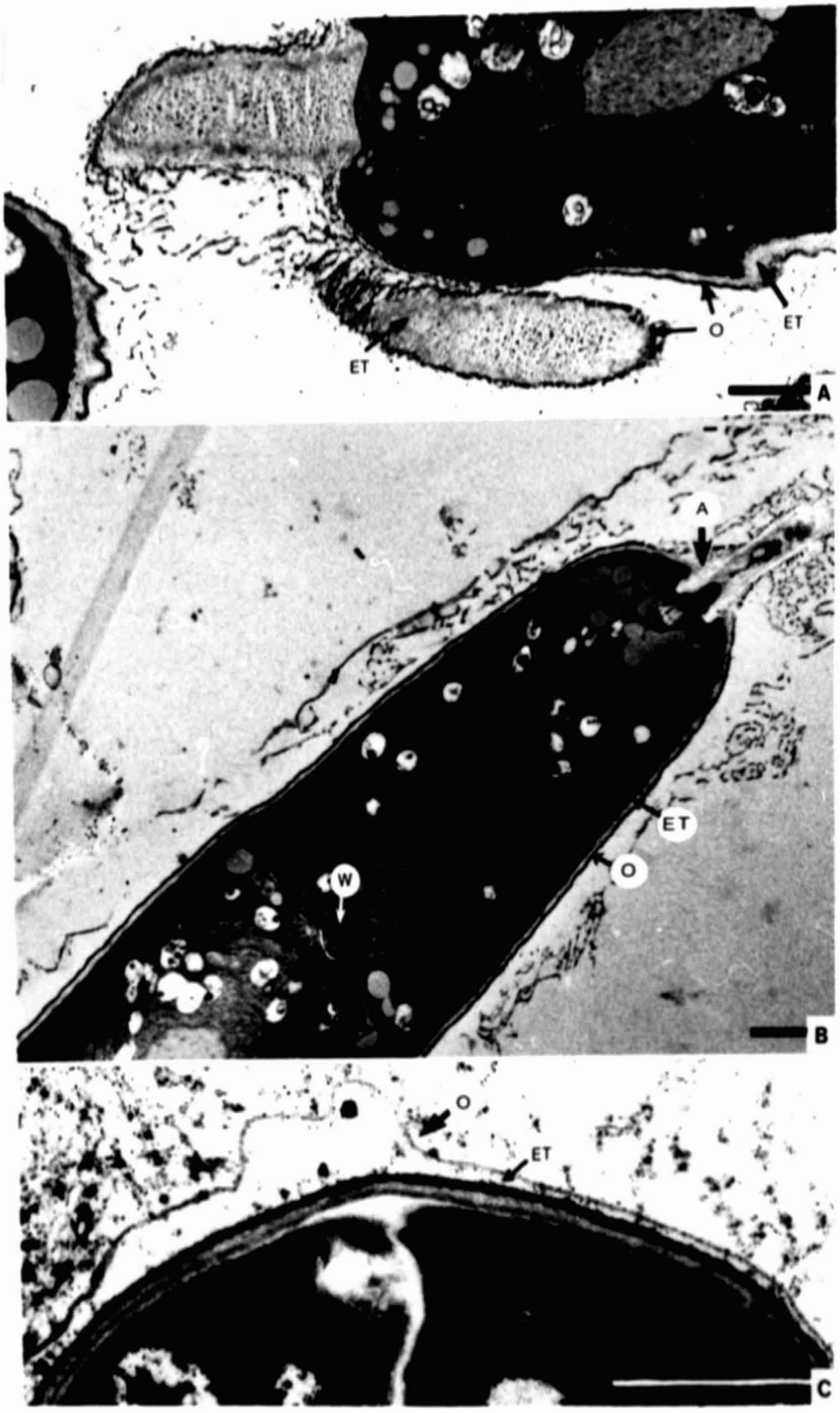

O
ET
ET
A
A
ET
O
W
B
O
ET
C

Fig. 12 Electron micrographs of the conidia of Urohendersonia platensis

A Adjacent conidia outside the pycnidium germinating to form anastomoses. Mucilage surrounds these conidia. (Bar = 1 µm)

B Germinating conidia outside the pycnidium forming anastomoses. Dead conidial cells devoid of cytoplasm can be distinguished. (Bar = 10 µm)

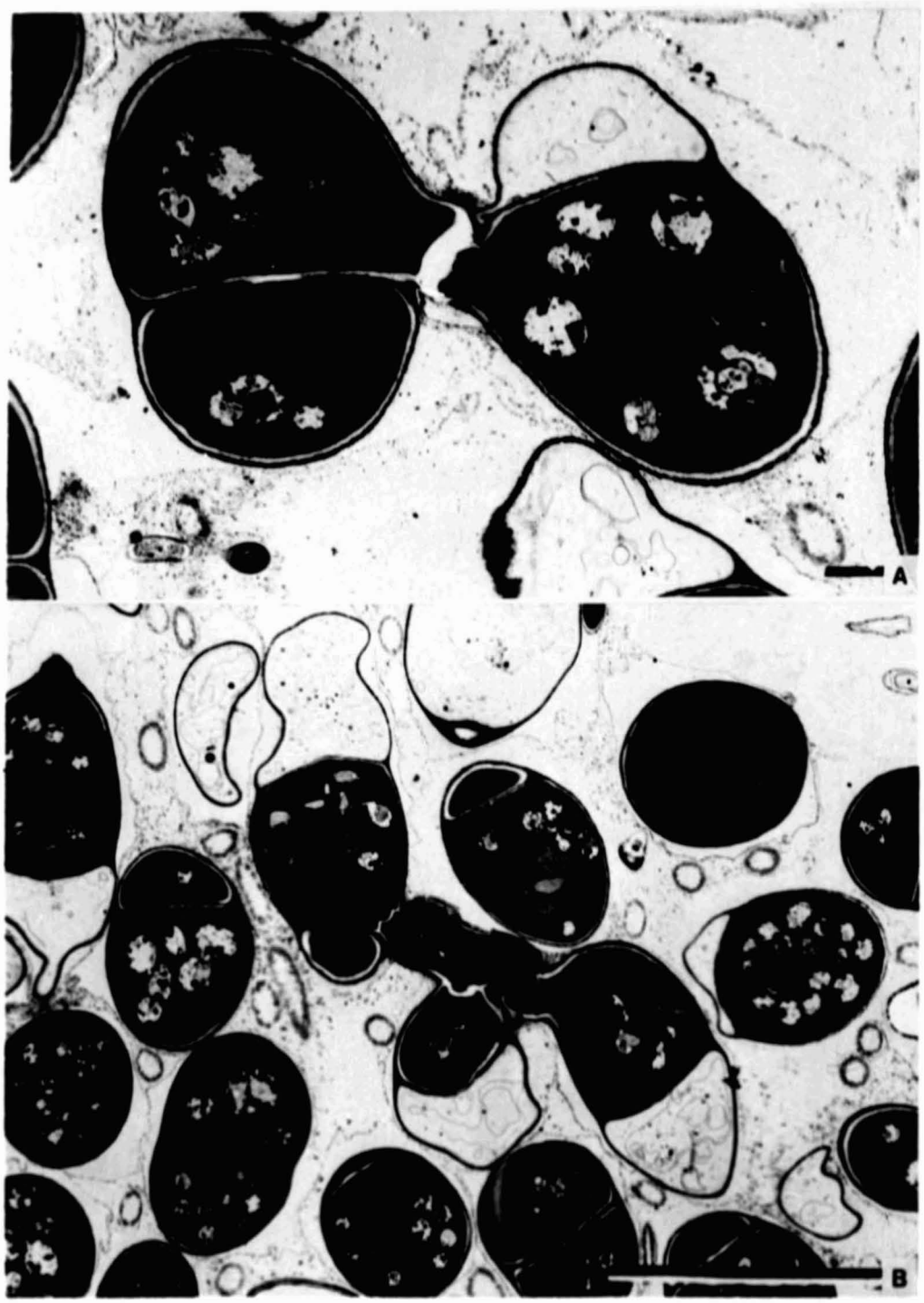

A
B

Fig. 13 Electron micrographs of conidia of Urohendersonia platensis

A Mature conidium showing the formation of mucilage from the outer osmiophilic and electron translucent layers. (Bar = 1 µm)

B Cells in a conidium start to fragment and the break on the one side has left a frill on both fragments, although the osmiophilic layer stays on the one cell (Bar = 1 µm)

C The break in the conidium has virtually gone through and remnants of the cell wall can be seen on either side of both fragments formed in this manner. The osmiophilic layer stays on one side (Bar = 1 µm)

C
B
A

Fig.14 Ontogeny of the conidioma of Tiarosporella graminis var karoo

a Young conidioma in vertical section showing lysis of the pseudo-parenchyma at four sites (Bar = 20 µm)

b Layer of conidiogenous cells showing underlying pseudoparenchyma and appendage formation (white halo) (Bar = 10 µm)

c Mature elongate conidiomata in vertical section (Bar = 100 µm)

d Detail of a locule showing conidiogenous layer and central lumen filled with conidia and mucus (Bar = 20 µm)

e Conidium with inverted apical appendage (Bar = 5 µm)

f Layer of fertile cells showing most stages of conidiogenesis and development of the apical appendage (Bar = 10 µm)

g Fat globules covering the conidiogenous cell layer (Bar = 10 µm)


a
b
c
d
e
f
g

Fig.15 Conidiogenesis in Tiarosporella graminis var karoo

a Conidiogenous cells covered with mucus and fat globules

b A part of the conidiogenous apparatus in a squashed preparation The underlying pseudoparenchyma, conidiophore and conidiogenous cells can be distinguished

c, d, e & f

Branched conidiogenous cells borne on varying conidiophores

g Conidia on lageniform conidiogenous cells

h, i & j

Conidia stained in monifled Leifson's stain showing detail of the apical appendage

k Conidium stained in erythrosin. The appendage is indistinct under interference contrast illumination. The guttulate nature of the conidium is, however, evident

l Conidium stained in erythrosin. The apical appendage is more distinct using bright field illumination

(All bars = 10µm).

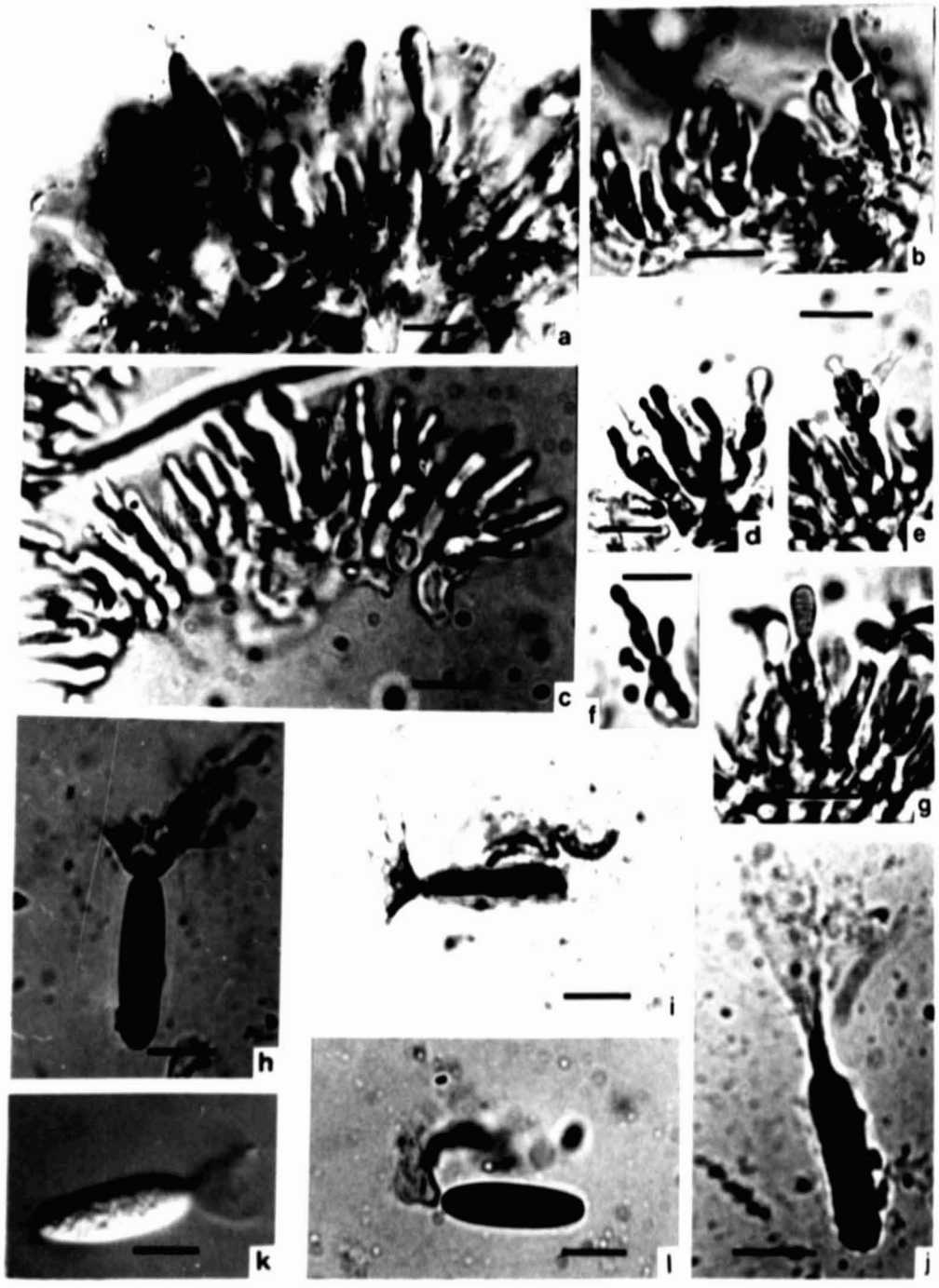

a
b
c
d
e
f
g
h
i
j
k
l

Fig. 16 Line drawings of conidiogenesis in Tiarosporella graminis var karoo

a Simple conidiogenous cells borne on a conidiophore

b - f

Branched conidiophore with conidiogenous cells producing conidia

g More intricate branching of a conidiophore with conidiogenous cells

h - j

More than one conidiogenous cell on each conidiophore

k - p

Conidia, some showing the veil-like apical appendage and guttulate nature of the body

(Bar = 10 µm)

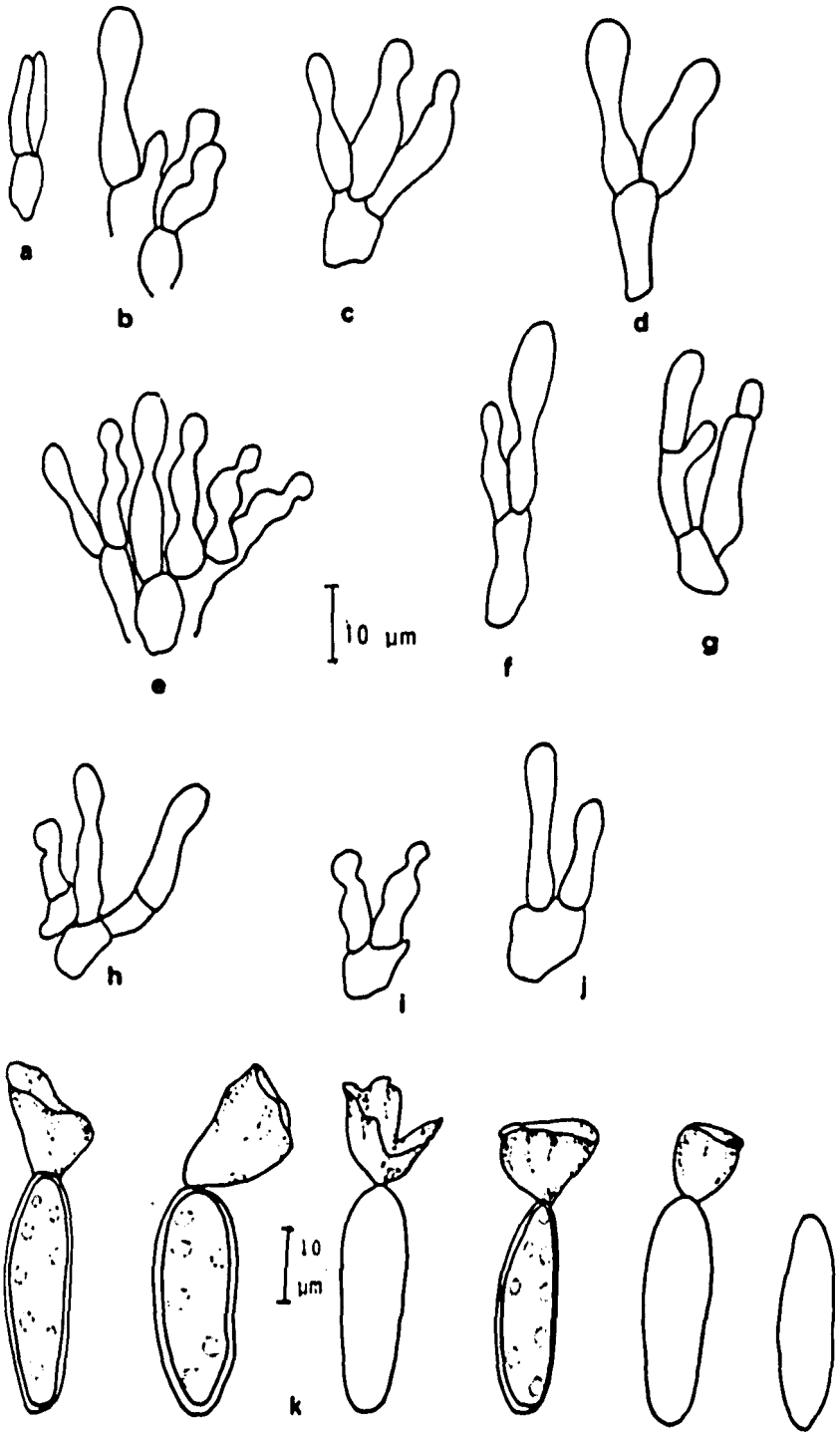

a
b
c
d
10 µm
e
f
g
h
i
j
10 µm
k

Fig. 17 Electron micrographs of Tiarosporella graminis var karoo

A Section through the pycnidial wall which consists of plectenchyma (PL), pseudoparenchyma (PP) and the conidiogenous apparatus (CA). The cells of the pseudoparenchyma are filled with mucus vesicles (MV). (Bar = 10 µm)

B Conidiogenous apparatus consisting of conidiophore (CP) bearing two conidiogenous cells (CC). (Bar = 1 µm)

C Conidiogenous cells producing conidia (C) which are enveloped in the incipient appendage (AA) with mucilage (M) covering the conidiogenous layer. The conidiogenous locus of the conidiogenous cell is indicated (CL). (Bar = 1 µm).

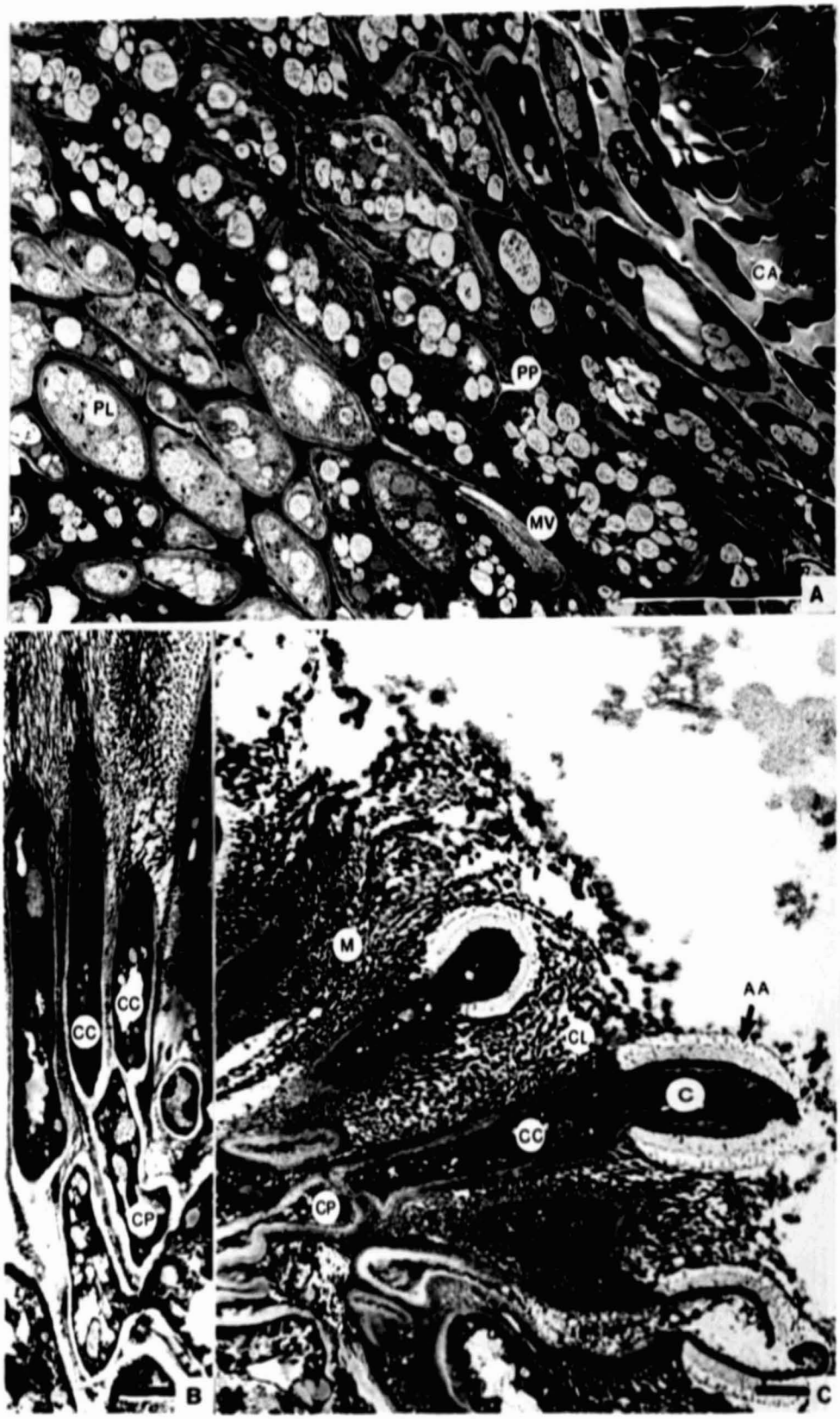

CA
PP
PL
MV
A
CC
CC
CP
B
M
AA
CL
C
CC
CP
C

Fig.18 Electron micrographs of Tiarosporella graminis var karoo

A Conidiogenous layer showing the osmiophilic conidiogenous cells in which a conidiogenous apparatus consisting of a conidiophore bearing two conidiogenous cells and a relatively mature conidium on one of these conidiogenous cells have formed the secession septum in the conidiogenous locus (CL). (Bar = 10 µm).

B Conidiogenous cell showing the holoblastic development of the conidium and the apical appendage which consists of a substance different from the surrounding mucus. The conidiogenous locus (CL) is arrowed. (Bar = 1 µm).

C Section through the conidiogenous layer showing the replacement of the conidiogenous apparatus through development of new cells (arrowed). The osmiophilic cells are the metabolically active meristem. The lighter coloured cells are underlying pseudoparenchyma. (Bar = 1 µm).


CL
A
CL
B
C

Fig. 19 Electron micrographs of Tiarosporella graminis var karoo

A Section through a conidium showing the osmiophilic and electron translucent cell wall and the electron translucent globular structures responsible for the guttulate appearance of the conidium under light microscopy. (Bar = 1 µm).

B Section through the pseudoparenchyma of the pycnidial wall showing the multitude of mucilage vesicles (MV) and a pore in the cell walls with the accompanying Woronin bodies arrowed (W). (Bar = 1 um).

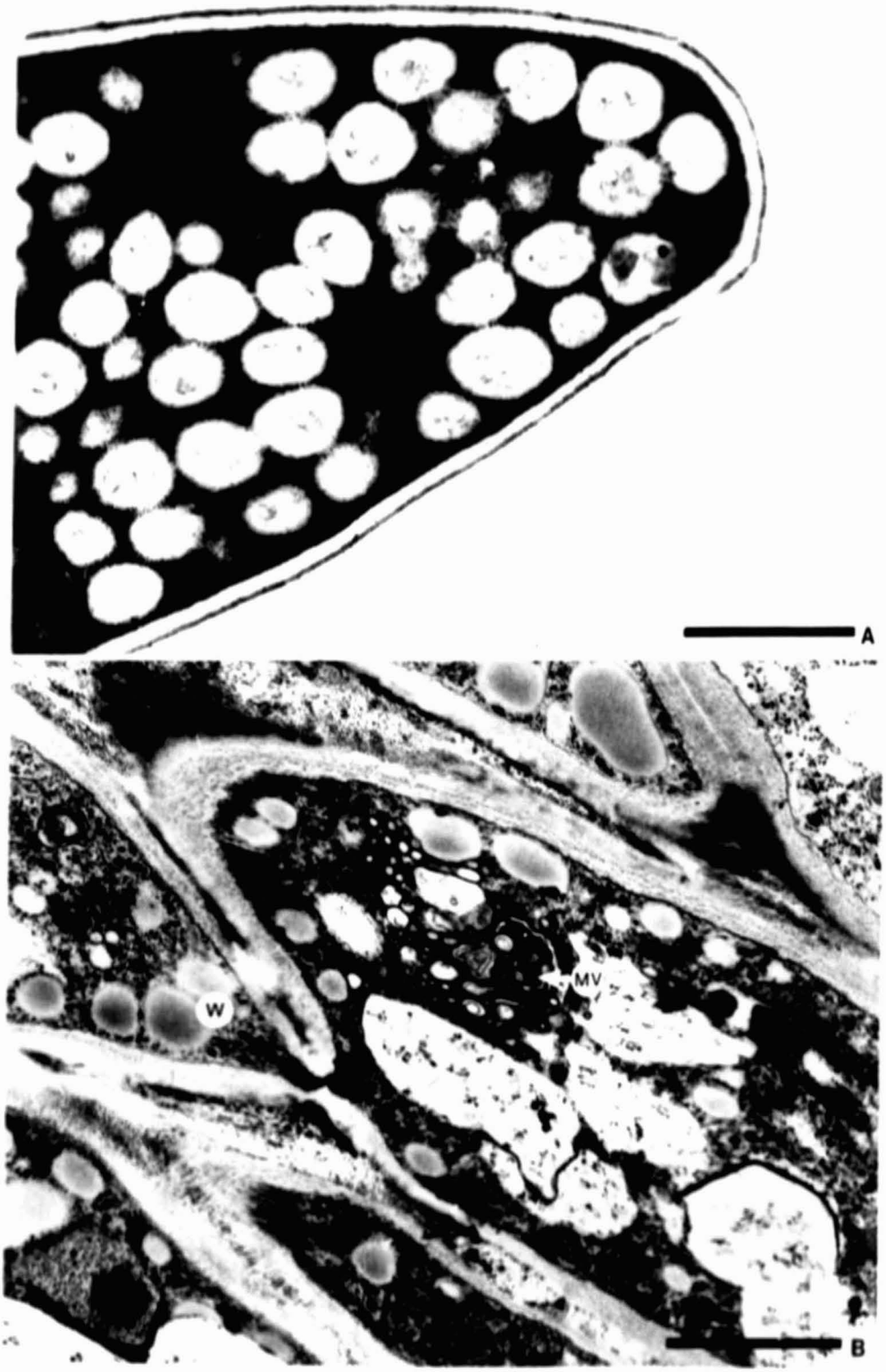

A
W
MV
B

Fig.20 Ontogeny of the conidioma of Bartalinia robillardoides

a Enlarged single hyphae (Bar = 10 µm)

b Additional hyphae crossing the first enlarged hyphae (Bar = 10 µm)

c Further concentration of convoluted enlarged hyphae to initiate formation of the conidioma (Bar = 10 µm)

d Pseudoparenchyma in central region of the young conidioma undergoing lysis prior to formation of the conidiogenous locules(Bar = 10 µm)

e The columns separating the locules become broken down by the transformation of the pseudoparenchyma into hyphal strands which bear the conidiogenous apparatus and from which conidiogenous cells arise (Bar = 50 µm)

f Two adjacent conidiogenous locules with layers of conidiogenous cells in horizontal section (Bar = 50 µm)

g Vertical section through a conidioma showing the darker plectenchyma of the vault (Bar = 50 µm)

h Lysis of the columnar pseudoparenchyma causing enlargement of the conidiogenous locule (Bar = 10 µm)

i Mature conidium showing the three apical cellular appendages and single basal appendage (Bar = 10 µm)

j Detail of the dead apical cell (Bar = 10 µm)

k Off-centre basal appendage. Note the length of the apical appendages in figs. i, k (Bar = 10 µm)

l A hyphal strand with ampulliform conidiogenous cells covered in mucilage and some fat globules (Bar = 10 µm)

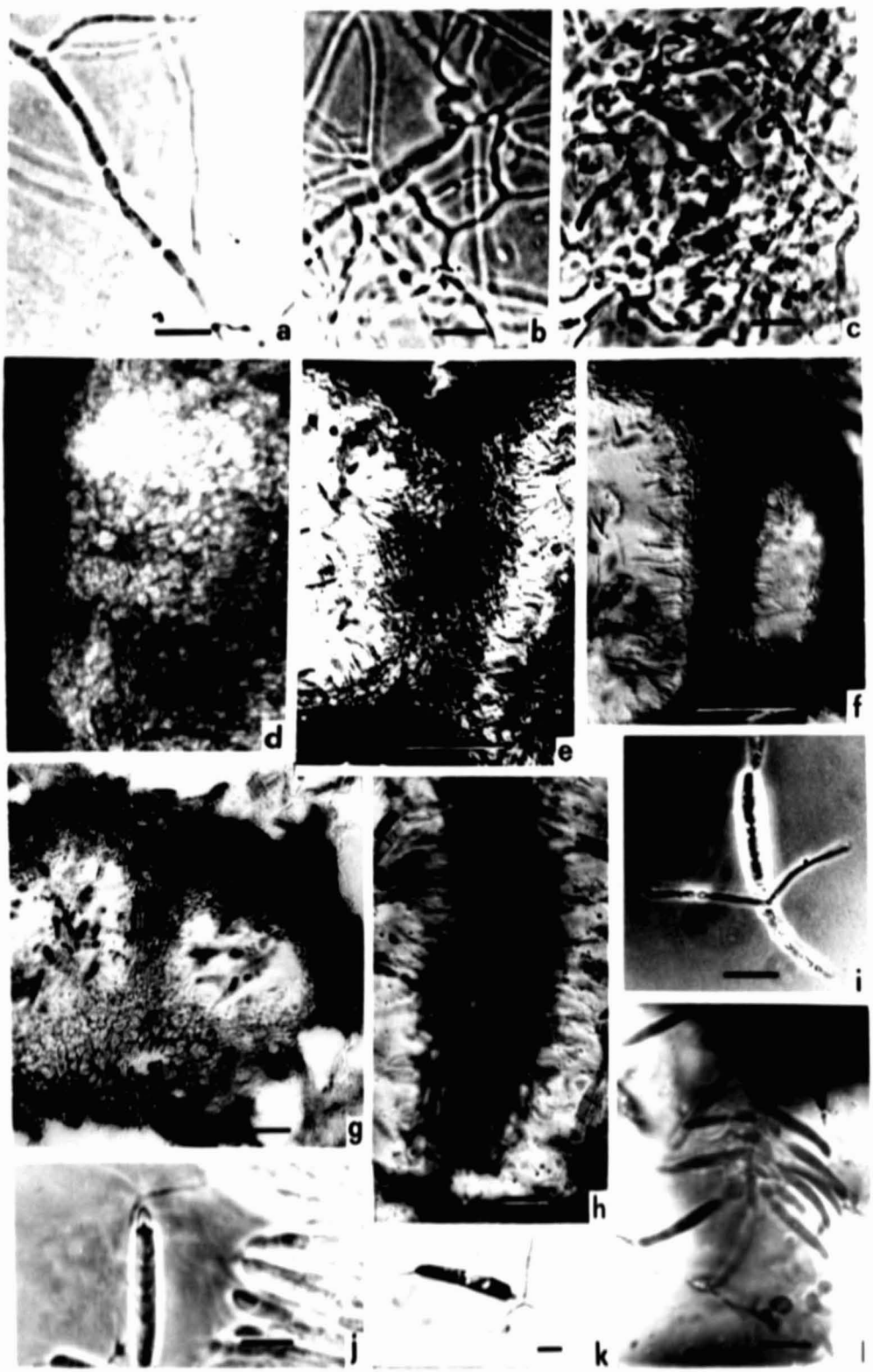

a
b
c
d
e
f
g
h
i
j
k
l

Fig.21 Conidiogenesis in *Bartalinia robillardoides*

a Hyphal strand bearing conidiogenous cells enveloped in mucilagenous material with surrounding fat globules

b Young conidium on a filiform conidiogenous cell, with developing apical appendage

c Conidiogenous cells on a branched conidiophore

d Conidium situated on a conidiogenous apparatus consisting of an intercalary cell, a conidiogenous cell and the conidiophore.

e An ampulliform and a filiform conidiogenous cell

f Complete conidiogenous apparatus with the elongated intercalary cell and young developing conidium

g A conidium attached to a complete conidiogenous apparatus where differentiation of the apical cell has taken place

h Conidia still attached to their conidiogenous apparatus with apical appendages in various stages of development

(All bars = 10 µm).

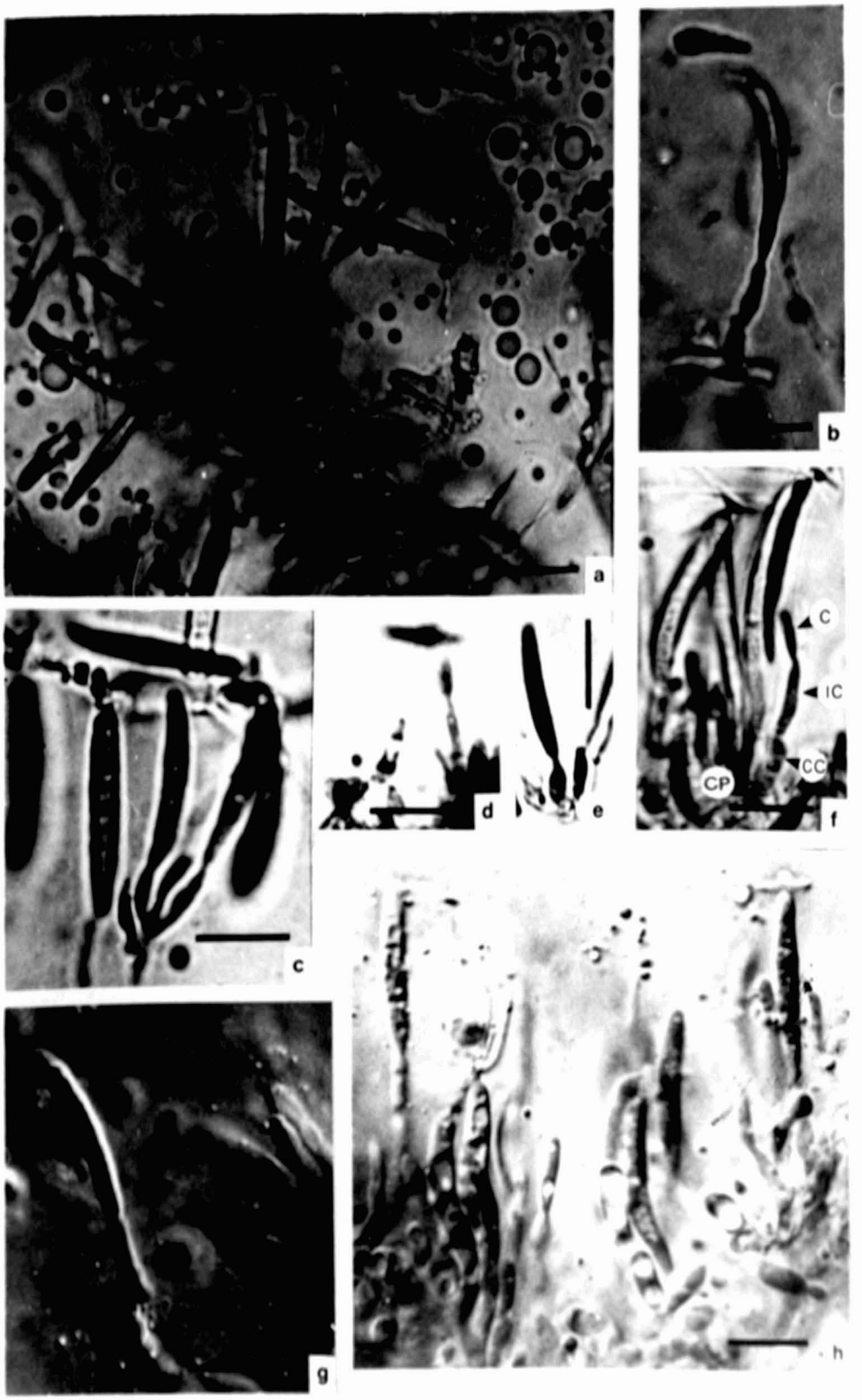

a
b
C
IC
CP
CC
f
d
e
c
g
h

Fig. 22 Conidioma of Bartalinia robillardoides in vertical plane

Serial sections of the conidioma from virtually the outside (a) through the various conidiogenous locules to the ostiole (h) and further. This series depicts the continuity between the various locules in the structure of the conidioma.

(Bar = 50 µm)

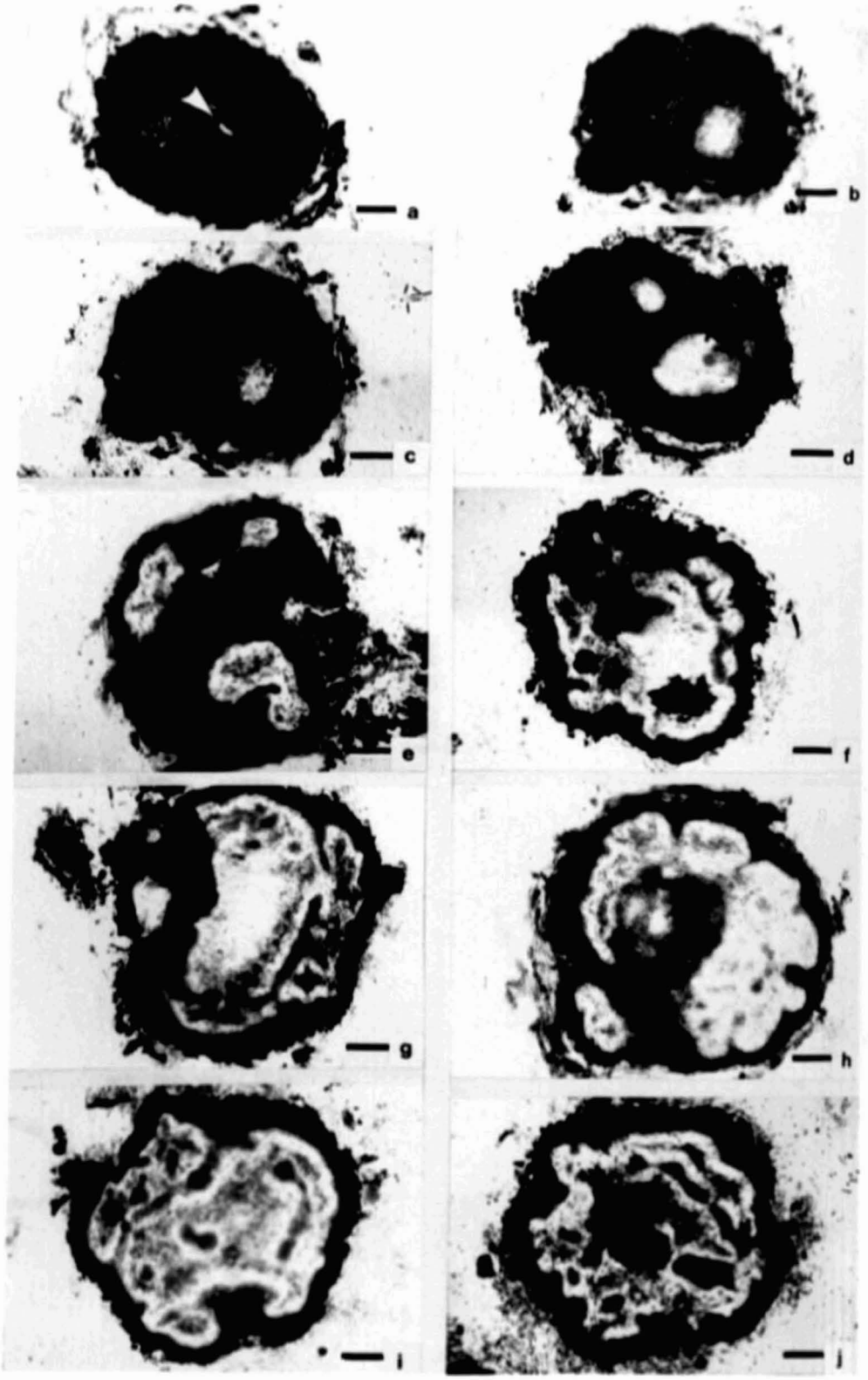

a
b
c
d
e
f
g
h
i
j

Fig. 23 Conidioma of Bartalinia robillardoides in horizontal plane

Serial sections of the conidioma in the horizontal plane, from the top showing the osiole (a), through the underlying locules etc., through all the various divisions which make up the multilocular structure to the the level of the agar substrate. This is an old conidioma.
(Bar = 50 µm)

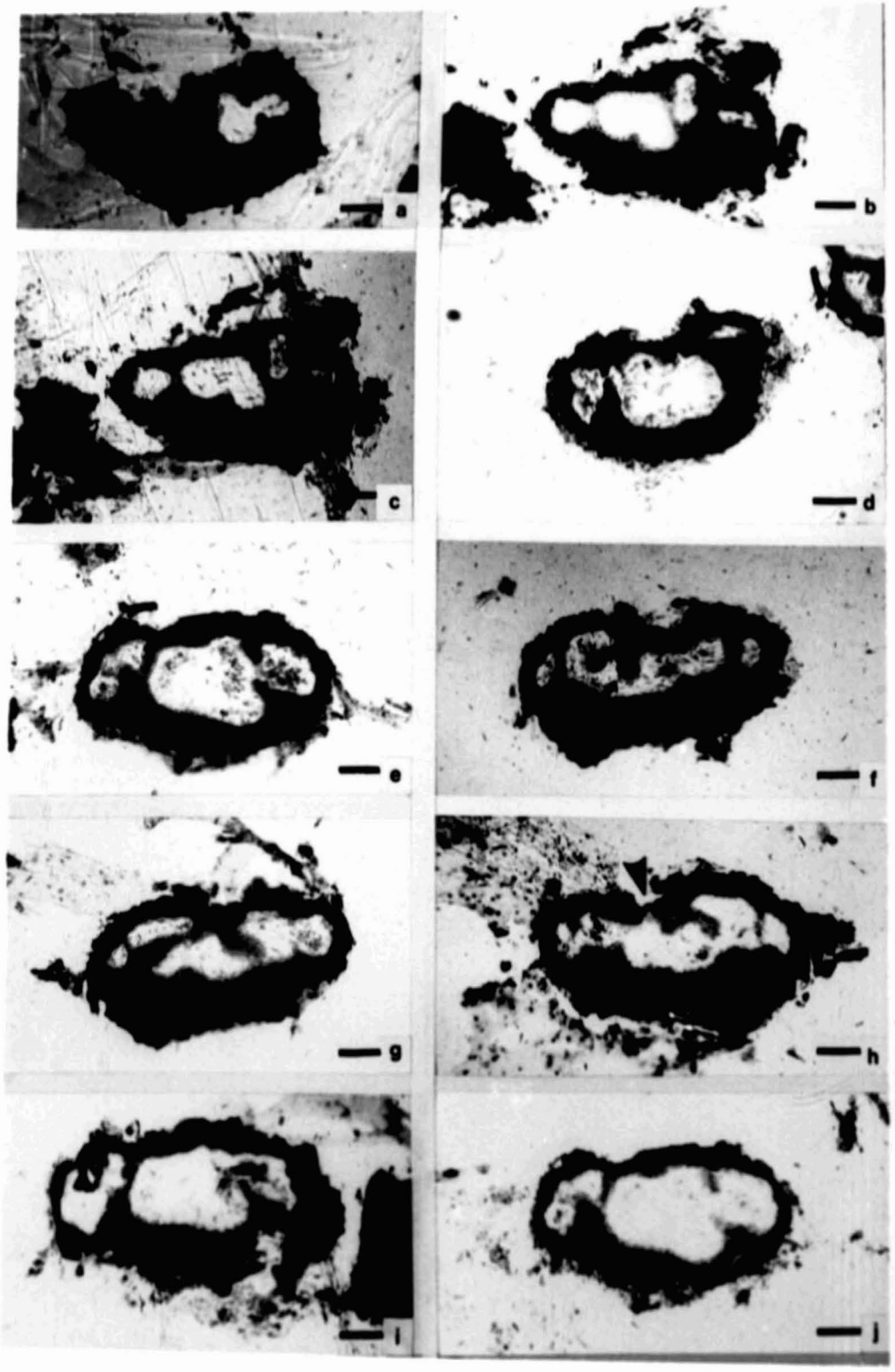

a
b
c
d
e
f
g
h
i
j

Fig.24 More detail of the conidioma of Bartalinia robillardoides

a Section through an Epon resin embedded conidioma, showing the multilocular connection at the ostiole. (Bar = 50 µm)

b The complete section shown in (a) showing the clypeate folds on top of the conidioma. (Bar = 50 µm)

c Section of a conidioma made by means of freeze microtomy showing the multilocular interior. (Bar = 50 µm)

d Detail of the ostiole in a freeze section where a disintegrating partition is shown (Bar = 50 µm)

e Conidiogenous cells in an old conidioma in which most conidia have been released. The conidiogenous apparatus has become much more elaborate and definite branching of the conidiophores and double annellations on the conidiogenous cells are indicated. (Bar = 10 µm)

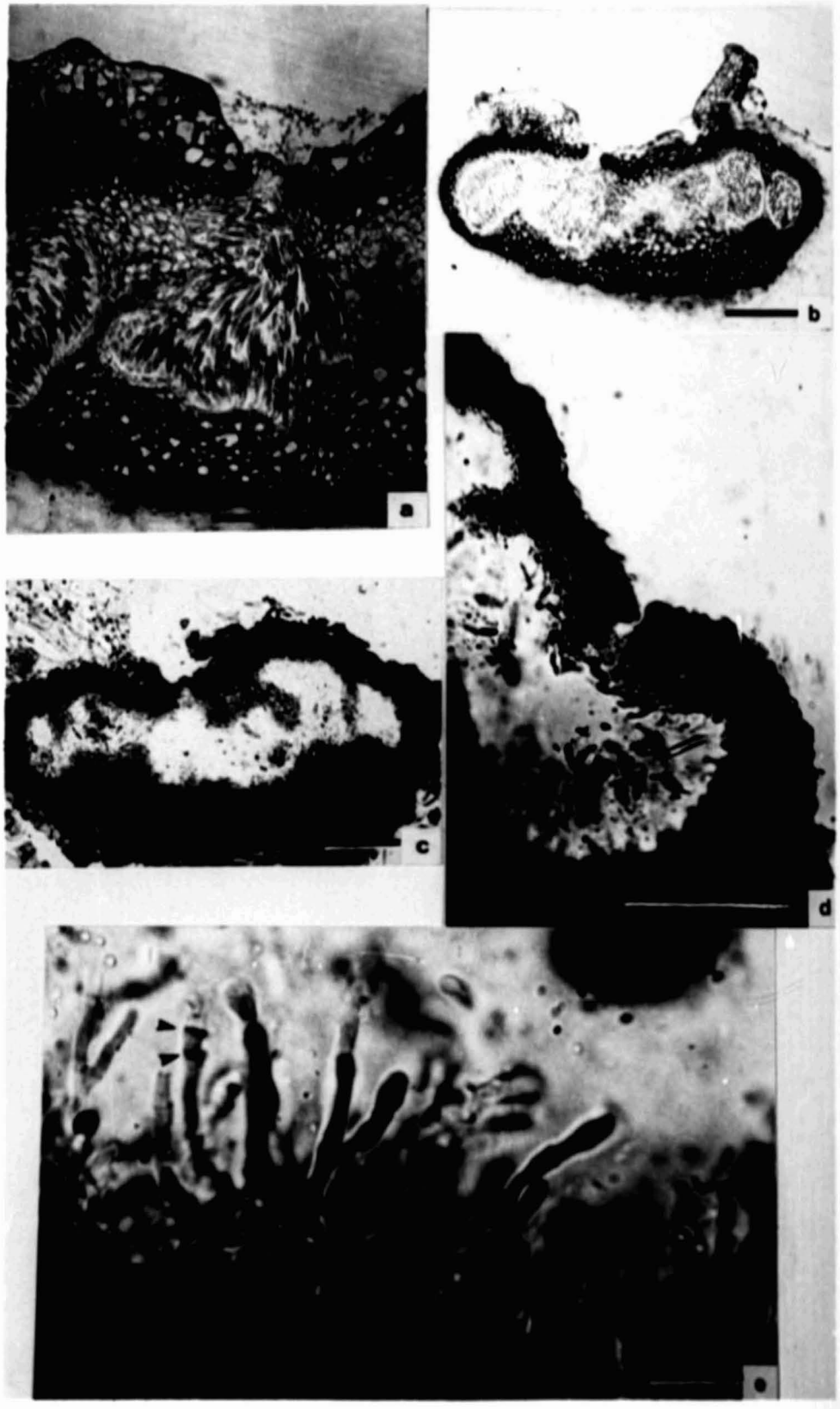
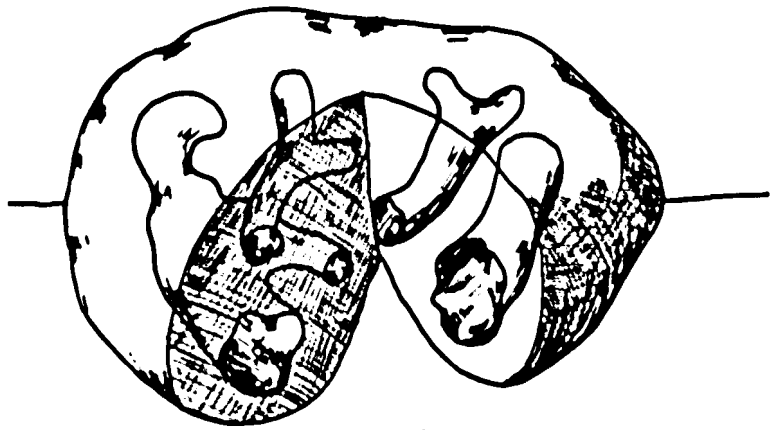
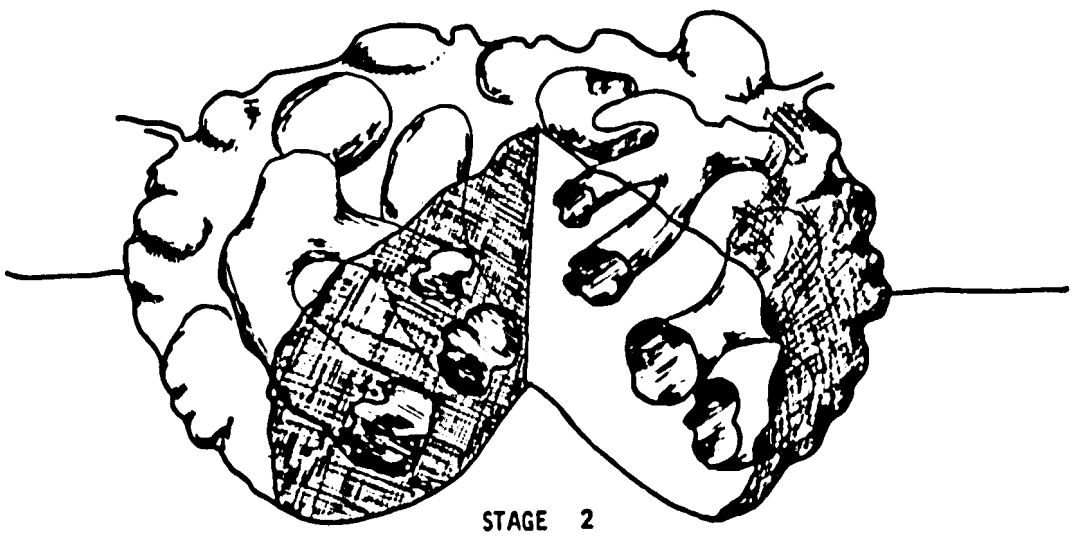

a
b
c
d
e

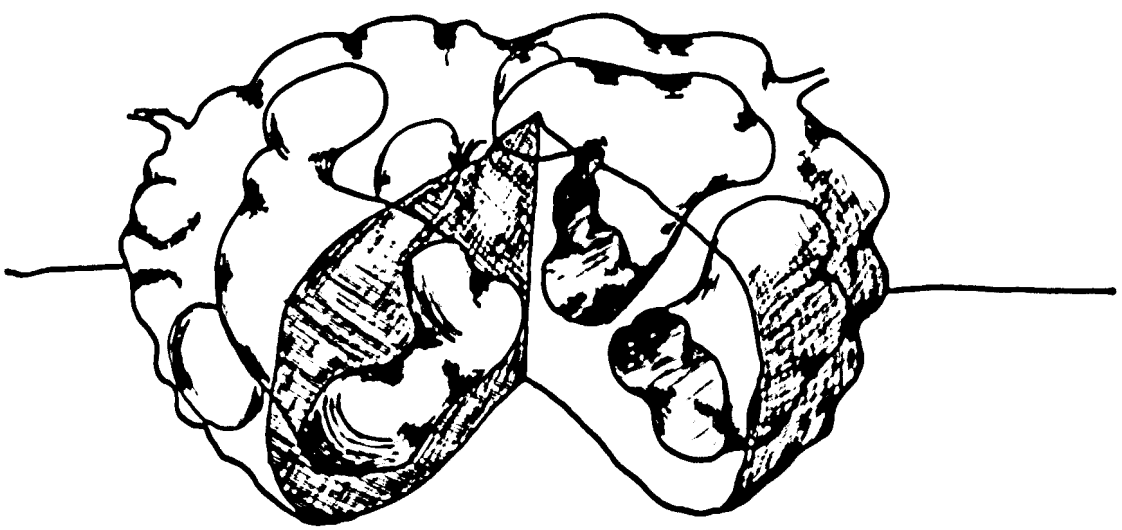
Fig. 25 Three dimensional drawings of the growth of the conidioma of Bartalinia robillardoides



STAGE 1



STAGE 2



STAGE 3

Fig. 26 Line drawings of *Bartalinia robillardoides*

a - h

Conidiogenous cells some of which are bearing conidia. (e) shows a conidiophore supporting the conidiogenous cell.

i Conidiogenous cells with what appears to be an annellation producing septate conidia with apical appendages

j A branched conidiophore with conidiogenous cells producing a conidium

k A branched conidiophore with an intercalary cell on the left

l Development of the three-armed appendage is shown. The conidium in the middle is supported by an intercalary cell.

m Conidia being produced on conidiogenous cells with an apparent annellation

n Conidiogenous cells with an apparent double annellation

o Four mature conidia with the fully developed basal and apical appendages

(Bar = 10 µm)

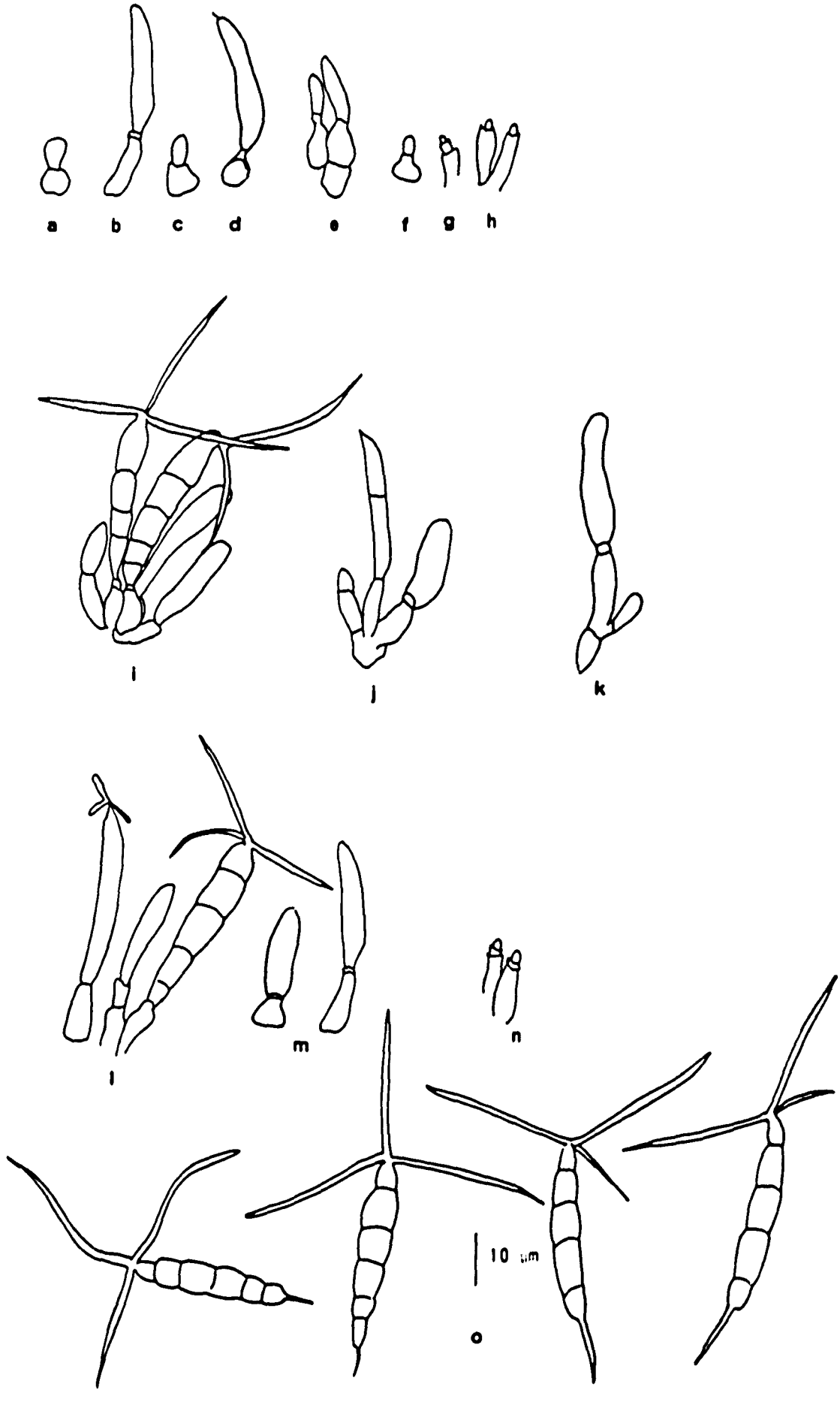

a
b
c
d
e
f
g
h
i
j
k
l
m
n
10 µm
o

Fig. 27 Electron micrographs of Bartalinia robillardoides

A Section through the pycnidial wall showing a conidiogenous cell with remnants of the rhexolytic secession as a frill at the apex (arrowed). Mucus vesicles in the pseudoparenchyma are indicated (MV). (Bar = 1 μm)

B Section through the conidiogenous layer showing development through replacement of dead cells by the next generation conidiogenous apparatus from the underlying pseudoparenchyma (Larger arrow). Dead conidiogenous cells (DC) dissolve to form mucilage, filling the conidiomal locule. (Bar = 1 μm).

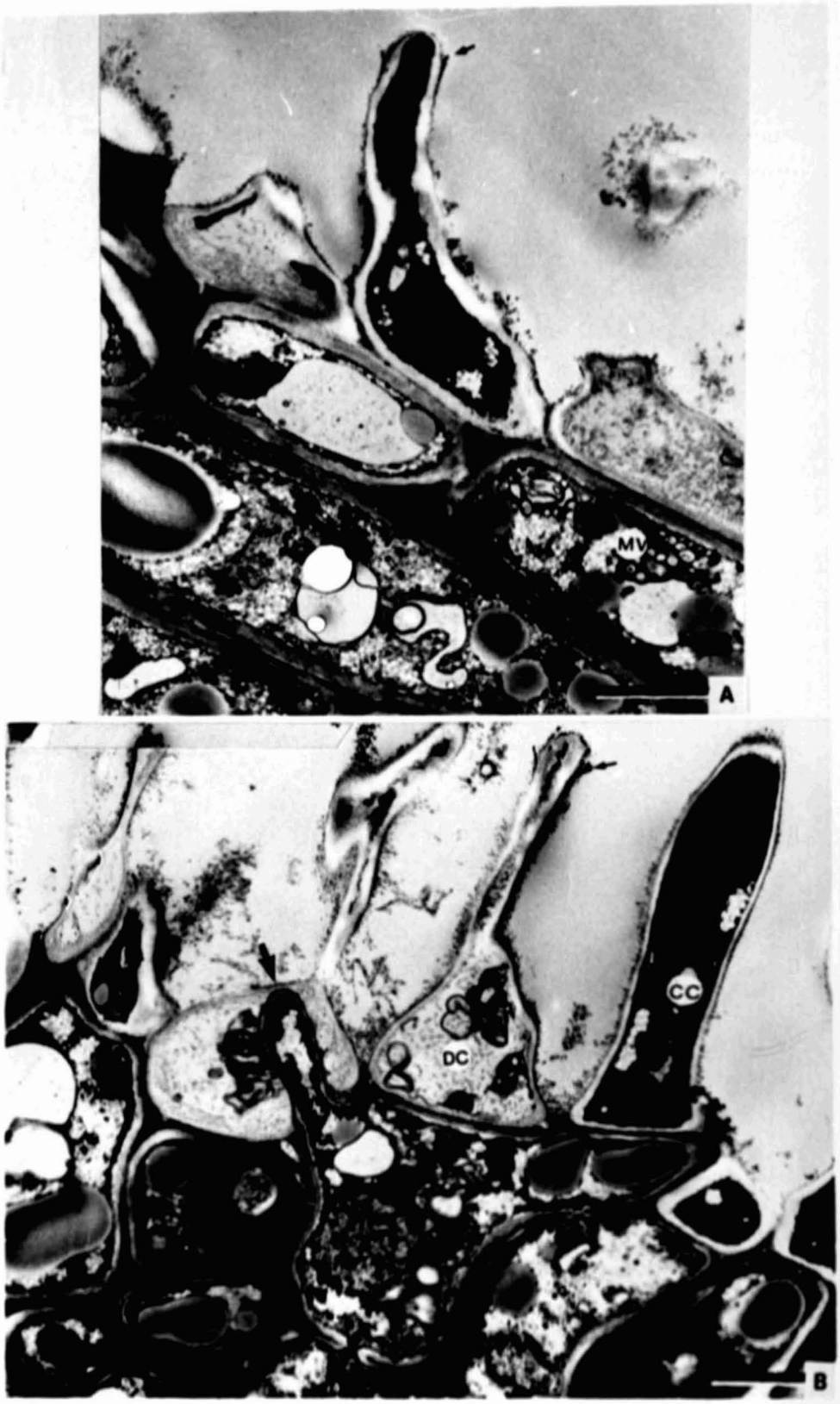

MV
A
CC
DC
B

Fig. 28 Electron micrographs of Bartalinia robillardoides

A Underlying pseudoparenchyma with uniform cell walls where lipids (L) secreted into the intercellular spaces to form an apparent osmiophilic layer between the cells are shown. (Bar = 1 µm)

B The conidiogenous apparatus showing the conidiophore (CP) and conidiogenous cells (CC), dead conidiogenous cells (DC) and mucilage forming the conidiogenous layer (Bar = 1 µm)

C Apical dead cell of the conidium, devoid of cytoplasm. An appendage in transverse section (A) is surrounded by mucilage (M). (Bar = 1 µm).


L
A
CC
CC
DC
CP
B
M
A
M
C

Fig. 29 Electron micrographs of Bartalinia robillardoides

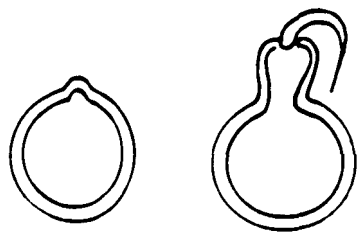
A Conidia showing the basal appendage in longitudinal section and a conidium in transverse section (circular). The basal appendage is formed by the electron translucent element of the conidial wall. (Bar = 1 µm).

B Another section through the basal appendage of the conidium showing the extended cell wall material (Bar = 1 um)

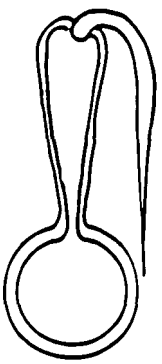
C Conidiogenous cell showing the osmiophilic remnants of the intercalary cell as a delicate frill at the level of the conidiogenous locus (arrowed) (Bar = 1 um).

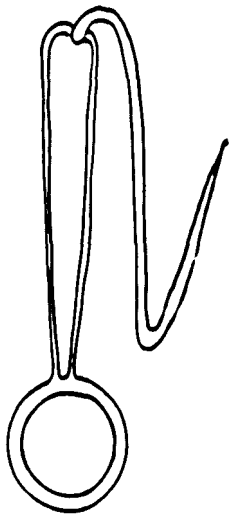

M
A
B
C

FIG 30 SCHEMATIC PRESENTATION OF THYSANOBLASTIC CONIDIOGENESIS AS WAS OBSERVED IN UROHENDERSONIA PLATENSIS

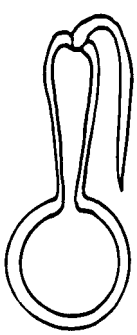


HOLOBLASTIC ONTOGENY OF FIRST CONIDIUM

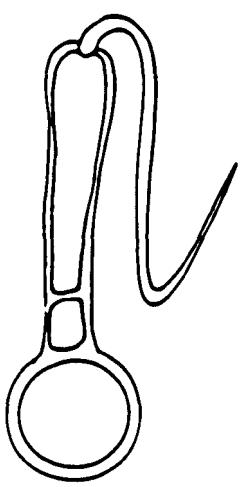


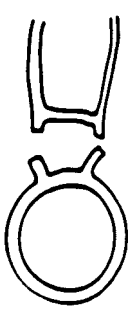


SCHIZOLYTIC SECESSION



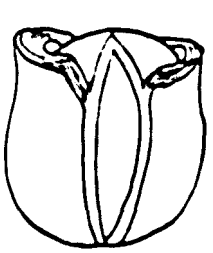
HOLOBLASTIC ONTOGENY OF THE SECOND CONIDIUM

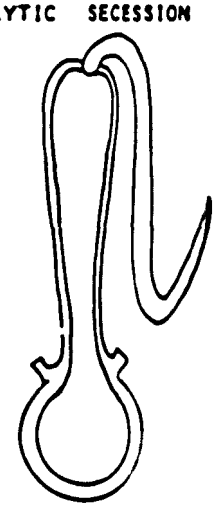




RHEXOLYTIC SECESSION

HOLOBLASTIC ONTOGENY OF THE THIRD CONIDIUM





SCHIZOLYTIC SECESSION

DEATH OF THE CONIDIOGENOUS CELL AND REPLACEMENT BY A NEW CONIDIOGENOUS CELL

FIG. 31. SYNOPSIS OF THE MORPHOLOGY AND CONIDIOGENESIS OF THE THREE COELOMYCETES.

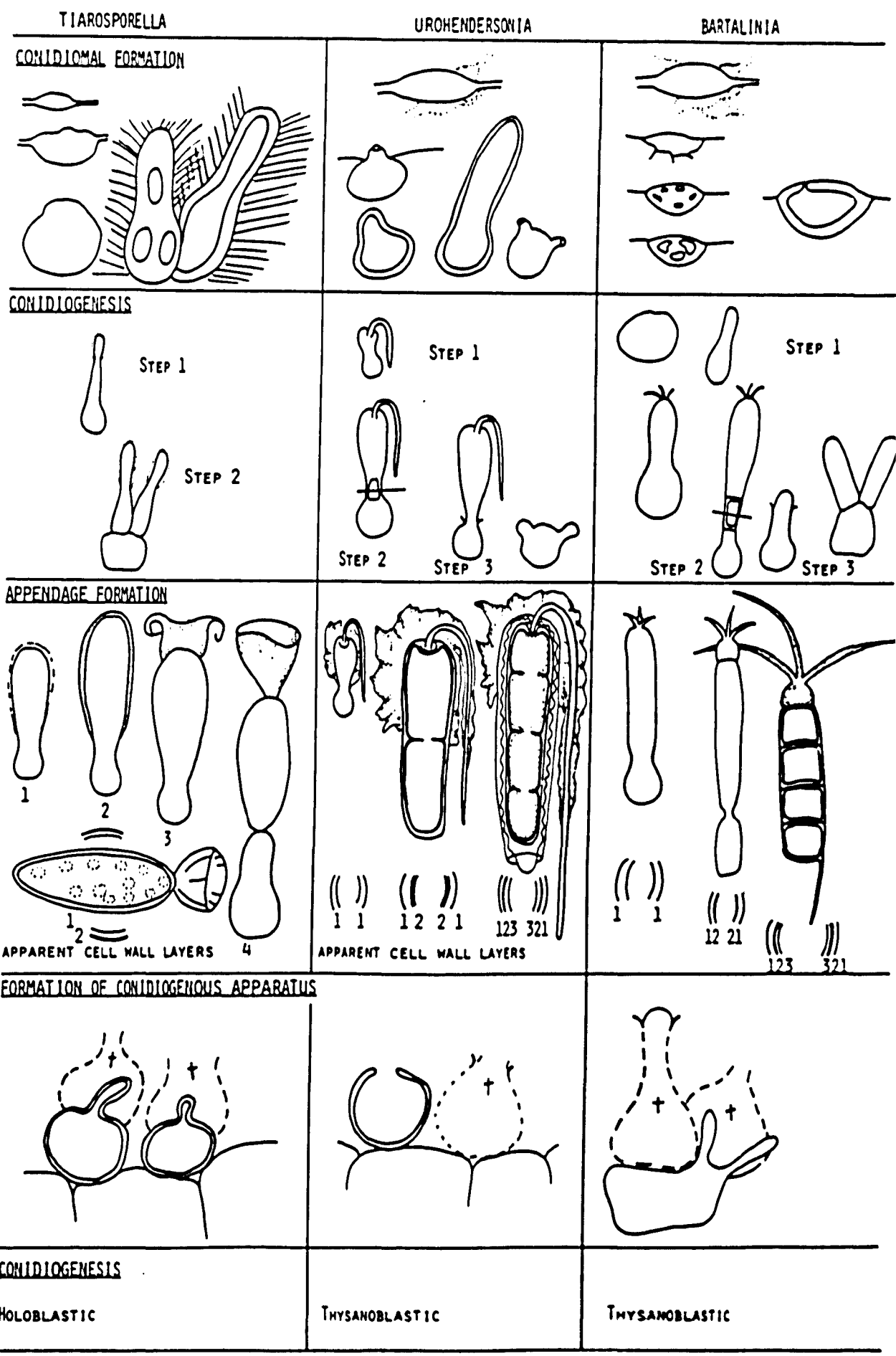


TABLE 1. ISOLATES OF THE THREE COELOMYCETES USED IN THE STUDY OF CONIDIOGENESIS.

PREM	IMI	LOCALITY	ISOLATED BY	IDENTIFIED BY	SUBSTRATE	DATE
Urohendersonia platensis Speg.						
47083	261321	Middelburg,C.P.	C. Roux	K.T. van Warmelo confirmed by B.C. Sutton	*Cynodon incompletus* Nees	Sept. 8
48040	265110	Brits, Transvaal	A.H. Thompson	C. Roux confirmed by B.C. Sutton	*Medicago sativa* L.	1980
48041	-	Middelburg, C.P.	C.Roux	C. Roux	*Felicia muricata* Thunb.	8.6.81
47854	-	Union Buildings Gardens, Pretoria	C. Roux	C. Roux	*Hedera helix* L.	March'85
Tiarosporella graminis (Pirozynski & Shoemaker) Nag Raj var *karoo* Sutton & Maresas						
48043	279783	Middelburg, C.P.	C. Roux	B.C. Sutton	plant litter	1980
47840	-	Steynsburg, C.P.	C. Roux	C. Roux	*Tribulus terrestris* L.	6.7.81
44967 Isotype	1186782	Colesberg, C.P.	W.F.O.Maresas	B.C. Sutton & W.F.O. Maresas	*Eriocephalus* L. sp.	Feb.1971
Bartalinia robillardoides Tassi						
48042	-	Lynnwood Park, Pretoria	C. Roux	C. Roux	associated with soil on *Ganoderma lucidum* (W.Curtis :Fr.) Karst.	Dec.'84
47110	-	Middelburg, C.P.	C. Roux	C. Roux	*Lycium cinereum* Thunb. *sensu lato*	Sept. 81

TABLE 2

Synopsis of the Morphology and Conidiogenesis of the three Coelomycetes in terms of the Literature

Urohendersonia platensis Speg.		*Tiarosporella graminis* (Piroz. & Shoemaker) var. *karoo* Sutton & Marasas		*Bartalinia robillardoides* Tass.: Morgan-Jones.	
Nag Raj & Kendrick (1971) Sutton (1980)	Present Study	Sutton & Marasas (1976) Sutton (1980)	Present Study	Nag Raj & Kendrick (1971) Sutton (1980)	Present Study
Conidiomata					
Pycnidia	Pycnidia	Pycnidia	Pycnidia	Pycnidia	Eustromatic
	Elongations	Rostrate	Elongated & Apically Rostrate	Unilocular	Multilocular becoming Unilocular
Ostiolate	Ostiolate, Multiostiolate A-ostiolate in immersed cultures	Ostiolate	Ostiolate	Ostiolate	Ostiolate
		Unilocular	Multilocular becoming Unilocular		
			Pycnidia basally coalescing		
Conidiophores					
Absent	Absent	Absent	Present simple & branched	Absent (However, Morgan-Jones, et al (1971) Fig. 4 illustrate conidiophores).	Present simple & branched
Conidiogenous cells, Conidiogenesis					
Holoblastic then annellidic	Thysanoblastic Conidiogenous cells could have 2 necks	Holoblastic	Holoblastic	Holoblastic then Annellidic	Thysanoblastic (percurrently thysanoblastic.
Conidia					
Occasionally Guttulate	Eguttulate	Eguttulate	Guttulate	Eguttulate	Eguttulate
Microconidia					
Not seen	Present	Absent	Absent	Absent	Absent
Conidial Appendages					
Apical Gelatinous Appendage	Apical appendage 1 inverted sheath 2 additional enveloping sheaths	Inverted envelope dividing into sections	Inverted envelope, Umbrella-like	3 apical & 1 off-centre basal cellular appendage	3 apical & 1 off-centre basal cellular appendage
Chlamydospores					
Absent	Present	Absent	Absent	Absent	Absent

REFERENCES

BARRON,G.L. (1968). *The Genera of Hyphomycetes from Soil*. Krieger: New York.

BOEREMA,G.H. & LOERAKKER,W.M. (1985). Notes on Phoma 2. *Transactions of the British Mycological Society* 84, 289 - 302.

BOEREMA,G.H.(1964). Phoma herbarum Westend., the type-species of the form-genus Phoma Sacc. *Persoonia* 3, 9 - 16, 2 plates.

CARROL,FANNY E. & CARROL, G.C. (1971). Fine structural studies on 'Poroconidium' formation in Stemphylium botryosum. In *Taxonomy of the Fungi Imperfecti*(Proceedings of the First International Specialists' Workshop-Conference on Criteria and Terminology in the Classification of Fungi Imperfecti held at the Environmental Sciences Centre of the Univeristy of Calgary, Kananaskis, Alberta, Canada) (Ed. B. Kendrick)pp. 75 - 91.Toronto:University Press.

COLE,G.T. & SAMSON,R.A. (1979). *Patterns of Development in Conidial Fungi*. Pitman:London.

COMMONWEALTH MYCOLOGICAL INSTITUTE (1983). *Plant Pathologists' Pocketbook*, 2nd edn.Kew:Commonwealth Agricultural Bureaux.

DI COSMO,F. & COLE,G.T. (1980). Conidial development in Chaetomella acutiseta (Coelomycetes). *Canadian Journal of Botany* 58, 1127 - 1137.

DODGE,B.O. (1930). Development of the asexual fructifications of Chaetomella raphigera and Pezizella lythri. *Mycologia* 22, 169 - 174.

ELLIS,M.B. (1971). *Dematiaceous Hyphomycetes*. Kew:Commonwealth Mycological Insitutute.

FISHER,N.L.,BURGESS,L.W.,TOUSSOUN,T.A. & NELSON,P.E. (1982). Carnation leaves as a substrate and for preserving cultures of Fusarium species. *Phytopathology* 72, 151 - 153.

HAWKSWORTH,D.L.,SUTTON,B.C. & AINSWORTH,G.C. (1983). *Ainsworth & Bisby's Dictionary of the Fungi* (7 ed).Kew: Commonwealth Mycological Institute.

HUGHES,S.J. (1953). Conidiophores, conidia and classification. *Canadian Journal of Botany* 31, 577 - 659.

HUGHES,S.J. (1971a). Percurrent proliferations in fungi, algae and mosses. *Canadian Journal of Botany* 49, 215 - 231.

HUGHES,S.J. (1971b). On conidia of fungi gemmae of algae, bryophytes and pteridophytes. *Canadian Journal of Botany* 49, 1319 - 1339.

KEMPTON,F.E. (1919). Origin and development of the pycnidium. *The Botanical Gazette* 68, 233 - 261. 5 plates.

KENDRICK,B. (1971) (ed.) *Taxonomy of the Fungi Imperfecti*. Toronto, Canada:University of Toronto Press.

KENDRICK,W.B. & CARMICHAEL,J.W. (1973). Hyphomycetes. In *The Fungi*, IV A (ed. G.C.Ainsworth,F.K.Sparrow & A.S.Sussman), p. 332. London, U.K.: Academic Press

LUTTRELL,E.S. (1963). Taxonomic criteria in Helminthosporium. *Mycologia* 55, 643 - 673.

MADELIN,M.F. (1979). An appraisal of the taxonomic significance of some different modes of producing blastic conidia. In *The Whole Fungus* 1 (ed. Bryce Kendrick), pp. 63 - 80. Ottowa, Canada : National Museum of Canada.

MARASAS,W.F.O., PAUER,G.D. & BOEREMA,G.H. (1974). A serious leaf blotch disease of groundnuts (Arachis hypogaea L.) in southern Africa caused by Phoma arachidicola sp.nov. *Phytophylactica* 6, 195 - 202.

MINTER,D.W.,KIRK,P.M. & SUTTON,B.C. (1982). Holoblastic phialides. *Transactions of the British Mycological Society* 79, 75 - 93.

MINTER,D.W.,KIRK,P.M. & SUTTON,B.C. (1983). Thallic phialides. *Transactions of the British Mycological Society* 80, 39 - 66.

MORGAN-JONES,G. & WHITE, J.F. (1983). Studies in the genus Phoma. III. Paraphoma. A new genus to accommodate Phoma radicina. *Mycotaxon* 18, 57 - 65.

MORGAN-JONES,G., NAG RAJ, T.R. & KENDRICK, B. (1972). Genera coelomycetarum. V. Alpakesa and Bartalinia. *Canadian Journal of Botany* 50, 877-882.

NAG RAJ,T.R. (1973). Genera coelomycetarum. X Ellisiella, Samukutu and Sakireeta. *Canadian Journal of Botany* 51, 2463 - 2472.

NAG RAJ,T.R. (1981). Coelomycete systematics. In *Biology of Conidial Fungi* I (ed. G.T. Cole & B. Kendrick). pp. 43 - 79. New York: Academic Press.

NAG RAJ,T.R. & KENDRICK,B. (1971). Genera coelomycetarum. I. Urohendersonia. *Canadian Journal of Botany* 49, 1853 - 1862.

PIROZYNSKI ,K.A. & SHOEMAKER,R.A. (1971). Some coelomycetes with appendaged conidia. *Canadian Journal of Botany* 49, 529 - 541.

PUNITHALINGAM,E. & WOODHAMS,J.E. (1984). A modified Leifson's flagella staining technique for revealing appendages in fungi. *Nova Hedwigia* 40, 31 - 43.

STOLK,AMELIA C. (1963). The genus Chaetomella Fuckel. *Transactions of the British Mycological Society* 46, 409 - 425.

SUBRAMANIAN,C.V. (1962). The classification of the Hyphomycetes. *Bulletin of the Botanical Survey of India* 4, 249 - 259.

SUTTON,B.C. (1968). Kellermania and its generic segregates. *Canadian Journal of Botany* 46, 181 - 196.

SUTTON,B.C. (1973). Coelomycetes. In *The Fungi* IV A (ed.G.C. Ainsworth,F.K.Sparrow & A.S.Sussman), pp. 513 - 582.

SUTTON,B.C. (1980). *The Coelomycetes*. Kew: Commonwealth Mycological Institute.

SUTTON, B.C. & MARASAS,W.F.O. (1976). Observations on Neottiosporina and Tiarosporella. *Transactions of the British Mycological Society* 67, 69 - 76.

SUTTON,B.C. & SANDHU,D.K. (1969). Electron microscopy of conidium development and secession in Cryptosporiopsis sp., Phoma fumosa, Melanconium bicolor, and M. apiocarpum. *Canadian Journal of Botany* 47, 745 - 549.

SUTTON,B.C. GHAFFAR,A. & ABBAS,S.Q. (1972). Seimatosporiopsis gen.nov. (Sphaeropsidales). *Transactions of the British Mycological Society* 59, 295 - 300.

TUBAKI,K. (1963). Taxonomic studies of Hyphomycetes. *Annual Report of the Institute for Fermentation, Osaka* 1, 25 - 54.

VOBIS,G. & HAWKSWORTH,D.L. (1981). Conidial lichen-forming fungi. In *Biology of Conidial Fungi* I (ed. G.T. Cole & B. Kendrick). pp. 245 - 271. New York : Academic Press.