A STUDY OF

SOUTH AFRICAN AQUATIC HYPHOMYCETES.

A thesis submitted to the

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by

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CONTENTS.

S U M M A R Y.

1. Eighteen species of aquatic Hyphomycetes are recorded from South Africa for the first time. All except two of these can be assigned to described species. The other two are new species of Articulospora and Anguillospora and are described in this thesis. Three unidentified spore types, an "Articulospora" type and "Anguillospora" type anda Y-shaped spore are also described.
2. Spore development in ten species is described.
3. A key to the fungi described in this thesis is given.
4. General notes on the ecology of these fungi and a table recording the fungi found in the Eastern Cape Province of South Africa, their distribution within the localities and the nature of the material on which they were growing are given.
5. The gross characteristics of plate cultures of six species, five growing on malt agar, Czapek agar, plain agar and maize agar and one on malt agar only are described.
6. Growth of four species in liquid culture using the Czapek-Dox medium is reported.
7. The results of an investigation into the effect of variations in temperature and medium on the growth of plate cultures of five species are analysed, discussed and conclusions are drawn.
8. Growth of certain isolates of four species on twigs is described.

INTRODUCTION.

The aquatic Hyphomycetes of South Africa have received little attention and therefore it seemed that an investigation of this group of fungi would be of value.

The aquatic Hyphomycetes flora seems to be dominated by a group of fungi which produce hyaline conidia, principally vermiform or branched in shape. The fungal nature of spores of the fungi of this aquatic group were apparently first recognised by De Wildeman (1893, 1894, 1895)[1] (see footnote) who described five species Tetracladium marchialanum De Wild.,[2] (see footnote) Fusarium elongatum De Wild. (now Anguillospora longissima (Sacc. & Syd.) Ingold), Clavariopsis aquatica De Wild. and Lemonniera brachycladia De Wild. He also figured conidia of other species (1893) together with a number of algae[3] (see footnote). Spores of Tetracladium setigerum (Grove) Ingold had been previously described and illustrated by Reinsch (1867 and 1882)[2] (see footnote). He however described it as Cerasterias raphidioides but expressed considerable doubt as to whether the organism in question was an alga or a fungus. De Wildeman noted the similarity of his fungus, Tetracladium marchialanum, to Cerasterias raphidioides Reinsch and also to Phycastrum longispina described and illustrated by Perty 1852[2] (see footnote). He submitted a sketch of the organism to Saccardo who expressed the opinion that it was undoubtedly a fungus and closely related to his Titaea callispora. De Wildeman reported it again in 1894[4] (see footnote) in France and Switzerland and classed it in the Phragmosporeae division of the family

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Footnote. 1. Cited by Ingold (1942)

2. Cited and illustrations reproduced by Karling (1935)
3. Cited by Nilsson (1958)
4. Cited by Karling (1935)

Mucedinaceae.

A long controversy over the identity of Tetracladium marchialanum followed and during its course the organism was confused with the algal genera Phycastrum, Polyedrium, Cerasterias and Asterothrix (reviewed by Karling 1935). By 1935 it had been recorded from Germany, France, Belgium, Switzerland, Africa, Norway, Dutch Guiana, Canada and the United States (Karling 1935) and from England (Barnes and Melville 1932). Karling (1935) observed spore development and by obtaining the organism in pure culture was able to clarify its nature and establish it as a fungus but he failed to recognise the existence of two species of Tetracladium, T. setigerum and T. marchialanum. His figures fit the description of T. setigerum (Grove) Ingold (Ingold 1942) rather than that of T. marchialanum De Wild.(Ingold 1942). He, however considers his fungus to be identical with Asterothrix (Cerasterias) raphidioides (Reinsch) Printz (1914)[1] which is clearly the spore of T. marchialanum. Tetracladium setigerum, which was first described as an alga by Reinsch (1888), was redescribed and drawn as a fungus, Tridentaria setigera, by Grove (1912) before it was finally placed in the genus Tetracladium by Ingold (1942). The only other aquatic Hyphomycete recognised prior to 1942 was Varicosporium elodeae Kegel in 1906. Apart from the six species mentioned above, none of the aquatic Hyphomycetes received any attention until 1942, when Ingold published an intensive study of the aquatic Hyphomycetes occurring in an alder lined stream near Leicester, England. From this site Ingold recognised sixteen species, six of which could be assigned to known species. Thirteen of these species were obtained in pure culture and spore development was followed in most of the species by using hanging drop cultures. Subsequent investigations by Ingold (1943 (a and b),

1944, 1952, 1958 and 1959), Ingold and Cox (1957) and Perrott (1960) have added a further fifteen British species. Virtually nothing was known of these fungi in North America, except for isolated accounts of Tetracladium marchialanum (Karling 1935) and Varicosporium elodeae (Bessey 1939) until Ranzoni (1953) made an intensive survey of the streams, lakes and ponds, both permanent and temporary, throughout the state of California. He recorded seventeen species which had been described by Ingold from Leicestershire, England, and an additional five new and undescribed species. One of these belonged to a new genus, Campylospora (Ranzoni). Tubaki (1957) made a survey of the streams, lakes and ponds of Japan and found that these fungi were common in well aerated water such as small streams and ponds. He described fourteen species, three of which were previously undescribed. In 1958 Tubaki recorded the occurrence of another four described species. The principal records of aquatic Hyphomycetes from Sweden are those of Willen (1958) who recorded nine described species and Nilsson (1958) twenty one described species, including the nine species recorded by Willen. In a collection of leaves from New Zealand streams Ingold (1959) identified seven described species. Hudson and Ingold (1960) and Ingold (1961) collected aquatic Hyphomycetes from streams in Jamaica and recorded sixteen species two of which were previously undescribed.

No intensive survey has been made of aquatic Hyphomycetes occurring in Africa. However, in the course of visits to Nigeria Ingold (1956, 1959 and 1961) has recorded spores of three described species and several undescribed species from collections of scum and foam from a stream. One spore of particular interest was branched and septate with clamp connections. Four described species and six types of spores of six

								outica											
X	X			X	X		X	X		X	X	X	X			X	X	X	Britain
								X			X	X	X				X	X	Scandina- via
																	X		Switzer- land
								X				X		X	X		X	X	California
											X								U.S.A.
		X				X								X			X	X	Jamaica
			X						X	X	X	X	X					X	Japan
						X								X				X	Nigeria
						X								X				X	Ghana
												X		X				X	Rhodesia
						X								X				X	Uganda

TABLE 1. A LIST OF THE DESCRIBED SPECIES OF AQUATIC HYPHOMYCETES AND AN INDICATION OF THE COUNTRIES FROM WHICH THEY HAVE BEEN RECORDED.

	Britain	Scandina-via.	Switzer-land.	California	U.S.A.	Jamaica	Japan	Nigeria	Ghana	Rhodesia	Uganda
Conidia hyaline											
Lunulospora curvula Ingold	X	X		X		X				X	X
Flagellospora curvula Ingold	X	X	X	X					X	X	X
Flagellospora penicillioides Ingold	X			X		X					
Anguillospora longissima (Sacc&Syd) Ingold	X	X		X		X	X				X
Anguillospora crassa Ingold	X					X	X				
Anguillospora flagellifera Ingold			X								
Anguillospora pseudolongissima Ranzoni				X							
Anguillospora gigantea Ranzoni				X							
Tricladium splendens Ingold	X	X	X	X			X				
Tricladium angulatum Ingold	X		X	X							X
Tricladium gracile Ingold	X			X							
Tricladium anomalum Ingold	X	X									
Heliscus lugdunensis Sacc & Therry (Heliscus aquaticus Syn)	X	X	X	X			X				
Heliscus longibrachiatus Ingold	X	X									
Heliscus stellatus Ingold & Cox	X	X									
Heliscus submersus Hudson						X					
Heliscus tentaculus Umphlett					X	X					
Clavariopsis aquatica Wild	X	X		X		X	X				X
Clavariopsis brachycladia Tubaki						X					
Tetracladium marchialanum Wild	X	X	X	X		X	X			X	X
Tetracladium maxilliformis (Rost) Ingold	X	X									
Articulospora tetracladia Ingold	X	X	X	X		X	X				X
Articulospora inflata Ingold	X	X					X				
Articulospora moniliforma Ranzoni				X							
Articulospora angulata Tubaki							X				
Alatospora acuminata Ingold	X	X	X	X		X	X			X	X
Tetracladium setigerum (Grove) Ingold	X	X	X			X	X				X
Triscelophorus monosporus Ingold											
Lemonniera aquatica De Wild											
Lemonniera brachycladia Ingold											
Lemonniera cornuta Ranzoni											
Campylospora chaetocladia Ranzoni											
Margaritispora aquatica Ingold											
Tetrachaetum elegans Ingold											
Varicosporium elodeae Kegel											
Dendrospora erecta Ingold											
Dactylella microaquatica Tubaki											
((Ingold)Ranzoni											
Dactylella aquatica (-Piricularia											
Piricularia submersa Ingold											
Actinospora megalospora Ingold											
Fluminispora ovalis Ingold											
Polycladium equiseti Ingold											
Speiropsis pedatospora Tubaki											
Jaculispora submersa Hudson & Ingol											
Tricellula aquatica Webster											
conidia dark brown											
Ankistrocladium fuscum Perrott											

																			Species	Habitat	Locality
X			X								X								Buddleja salvifolia	Natural Forest.	HOGSBACK
			X					X			X	X		X		X		X	Rubus sp.		
							X												Plectranthus ecklonii		
	X		X		X						X							X	Celtis africana		
																X		X	Xymalos monospora		
X					X						X					X			Rhus legati		
			X								X								Grewia occidentalis		
			X		X			X	X	X	X	X	O	X	X		X	X	Maytenus cymosus		
			X																Calodendrum capensis		
X															X	X			Fagara davyi		
		X	X								X					X			Podocarpus latifolius		
			X																Polystichum sp.		
					X						X				X				Royena lucida	Fynbos	
											X					X			Cassine sp.		
					X						X	X							Rubus sp.		
							X											O	Salix babylonica	Baakens River.	PORT ELIZABETH
									O					X					Populus sp.		

TABLE 2. TABLE OF THE AQUATIC HYPHOMYCETES FOUND IN THE EASTERN CAPE PROVINCE OF SOUTH AFRICA AND THE NATURE OF THE MATERIAL ON WHICH THEY WERE GROWING.

	GRAHAMSTOWN DISTRICT																									ALICE			
	Fern Kloof.	Feather Stone Kloof	Belmont Valley	Paradise Kloof							Howlesons Poort						Orinway		Rabbit Bush				Tyumie	River.	Plantation.		Mixed Plantation.	
	Urtica sp.	Rhus legati	Quercus robur	Populus nigra	Quercus robur	Octea bullata	Cassine sp.	Canthium hispinosum	Celtis africana	Rapanea melanophloeos	Podocarpus latifolius	Rhus lucati	Unidentified leaf	Rapanea melanophloeos	Burchellia bubalina	Ficus capensis	Hartephyllum caffrum	Populus nigra	Quercus robur	Rhus lucati	Celtis africana	Curtonia capensis	Acacia sp.	Combretum caffrum	Salix babylonica	Populus serotina (?P.nigra x deltoides).	Quercus robur	Eucalyptus globulus	Acacia sp.decaying fruit
Lunulospora curvula Ingold			X	X		X	X		X	X		X		X		X	X			X	X	X	X		X	X	X	
Flagellospora penicillioides Ingold				X																									
Flagellospora curvula Ingold																								X		X	X		
Anguillospora filiformis n.sp.		X						O	O	X		X										X				X	X	X	
Anguillospora longissima (Sacc & Syd) Ingold				X																						X			
Anguillospora sp.																													
Tricladium splendens Ingold																										X	X		
Tricladium gracile Ingold				X																	X					X	X	X	
Heliscus longibrachiatus Ingold																										X	X	X	
Clavariopsis aquatica De Wild				X	X														O							X	X		
Tetracladium setigerum (Grove) Ingold		X																								X			
Tetracladium marchialanum De Wild				X	X																		X			X	X		
Articulospora sp.													X													X			
Articulospora tetracladia Ingold	X	X	X						X	X	X	X	X													X	X		
Articulospora grandis n.sp.									X	X	X			X							X								
Alatospora acuminata Ingold	O								X			X		X	X	X							X		X	X	X	X
Triscelophorus monosporus Ingold				X					O		X	X		X	X		X	X	X				X		X		X		
Lemonniera aquatica De Wild																										X	X		
Lemonniera brachycladia Ingold																										X			
Camptospora chaetocladia Ranzoni																													

undescribed species have been recorded from scum and foam of streams in three localities in Ghana (Dixon 1959). Ingold (1958) recorded thirteen described species of aquatic Hyphomycetes and spores of a number of undescribed species from Uganda and Southern Rhodesia. A list of the described species of aquatic Hyphomycetes and an indication of the countries from which they have been recorded are given in Table 1.

These fungi have been found growing on a wide range of decaying plant material. Attention has been drawn to the fact that all gymnosperm material examined was devoid of these fungi (Ingold 1959 and Nilsson 1958). It is therefore of interest that the decaying leaves of a gymnosperm, Podocarpus latifolius, have been found in the present survey to be inhabited by Flagellospora curvula, Tricladium gracile, Articulospora tetracladia, Articulospora grandis n. sp., Alatospora acuminata and Triscelophorus monosporus. Table 2 records the aquatic Hyphomycetes found in the present survey in the Eastern Cape Province of South Africa and the nature of the material on which they were growing.

The aquatic fungi of this group are usually classified in the Fungi Imperfecti, as the perfect stage was for a long time unknown and is still only known in two species, Flagellospora penicillioides (Ranzoni 1953) and Heliscus lugdunensis (Webster 1959), both of which are species of Nectria. In this investigation an attempt was made to obtain the perfect stages by growing certain isolates on sterilised twigs.

Additional forms of reproductive bodies have been described for some species, for example pycnidiospores of Clavariopsis aquatica (Ingold 1942), small spherical or ovoid phiallospores produced by a number of species (e.g. Tricladium gracile (Ranzoni 1953) and Heliscus

lugdunensis (Webster 1959)), chlamydospores formed by Flagellospora penicillioides (Ingold 1944) and sclerotia formed by some species such as Clavariopsis aquatica (Ingold 1942).

Little experimental work has been carried out on aquatic Hyphomycetes. Webster (1959) investigated the sedimentation and impaction of the spores on smooth surfaces. He presented evidence for the evolution of tetraradiate propagules in a number of unrelated organisms as a probable adaptation to an aquatic environment and discussed speculations about the significance of such structures. From sedimentation experiments he observed that tetraradiate spores did not separate more rapidly than other spores. However, he found that the trapping efficiency of tetraradiate spores was considerably higher than that of the other spore forms studied (e.g. Anguillospora longissima and Flagellospora sp.)

Tubaki (1957) studied the relationship between growth and sporulation, and temperature in thirteen species. From these results he concluded that the limit of growth for these species was about 25-27°C. on solid media while that of sporulation in liquid culture was 25°C.

In this investigation a survey was made of the aquatic Hyphomycetes occurring in the Eastern Cape Province of South Africa. Eighteen species of aquatic Hyphomycetes were found and are recorded from South Africa for the first time. All except two of these can be assigned to described species and the other two are a new species of Articulospora and Anguillospora and are described in this thesis. Three unidentified spore types, an "Articulospora" type, an "Anguillospora" type and a Y-shaped spore were also found. A key was made to these fungi, observations were made on their

ecology and spore development was followed in eleven species. Six species were isolated in pure culture, four of these were grown in liquid culture in Czapek-Dox medium and an investigation was made into the effect of five temperatures and four media on the growth of plate cultures of five species. These isolates were also grown on twigs in the hope of obtaining the perfect stage, as both species of which the perfect stage is known are Nectria spp., and Nectria spp. are common on twigs in the localities in which aquatic Hyphomycetes were collected in the present investigation.

PART 1.

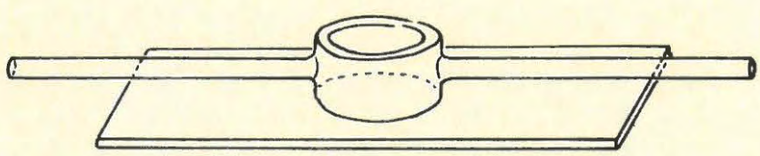
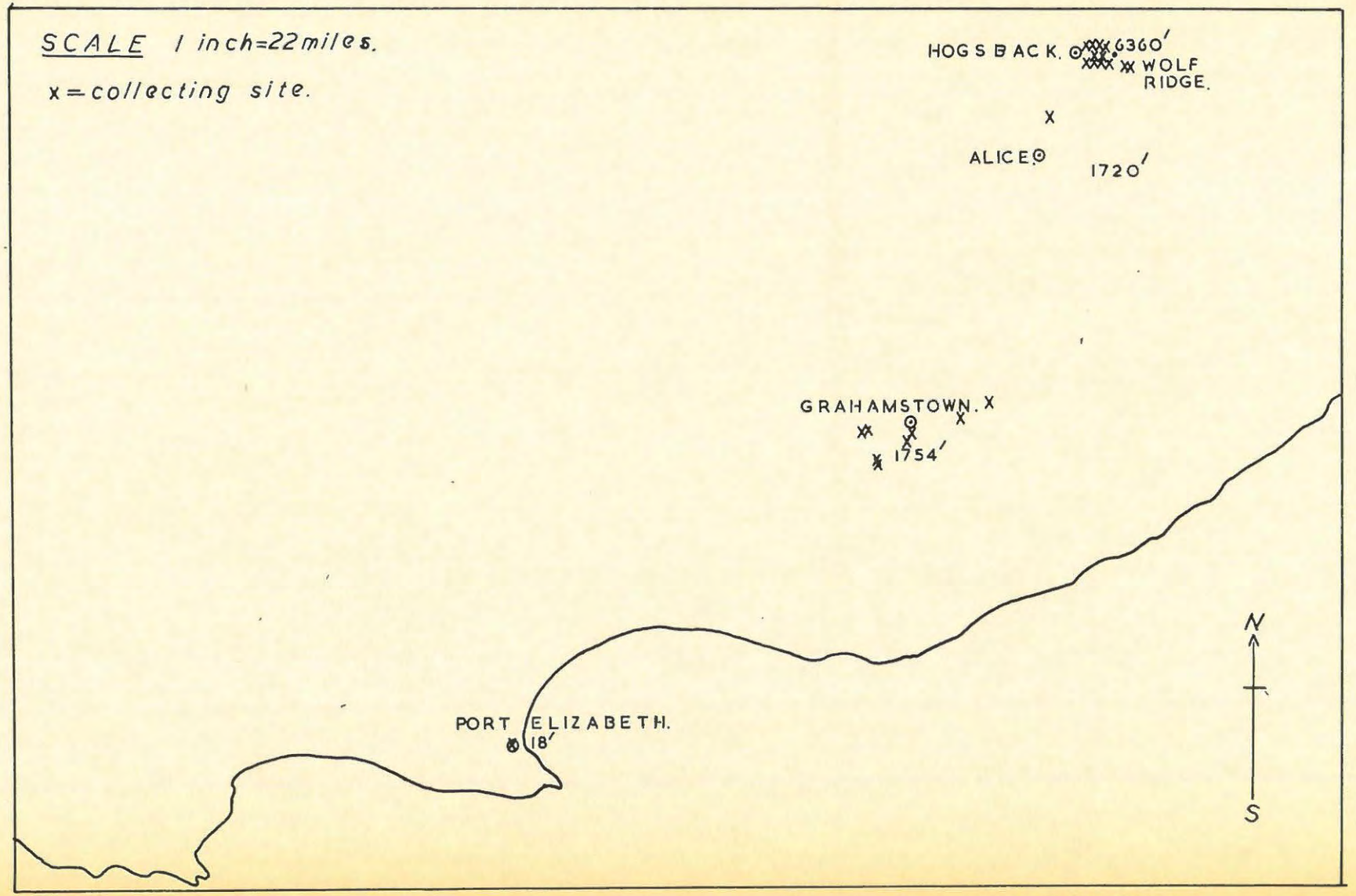


Fig.1. Slide bearing ring-chamber with an inlet and and an outlet tube. Used for hanging-drop cultures.



MAP I.

MAP OF MAIN COLLECTING AREAS IN THE EASTERN CAPE PROVINCE OF SOUTH AFRICA

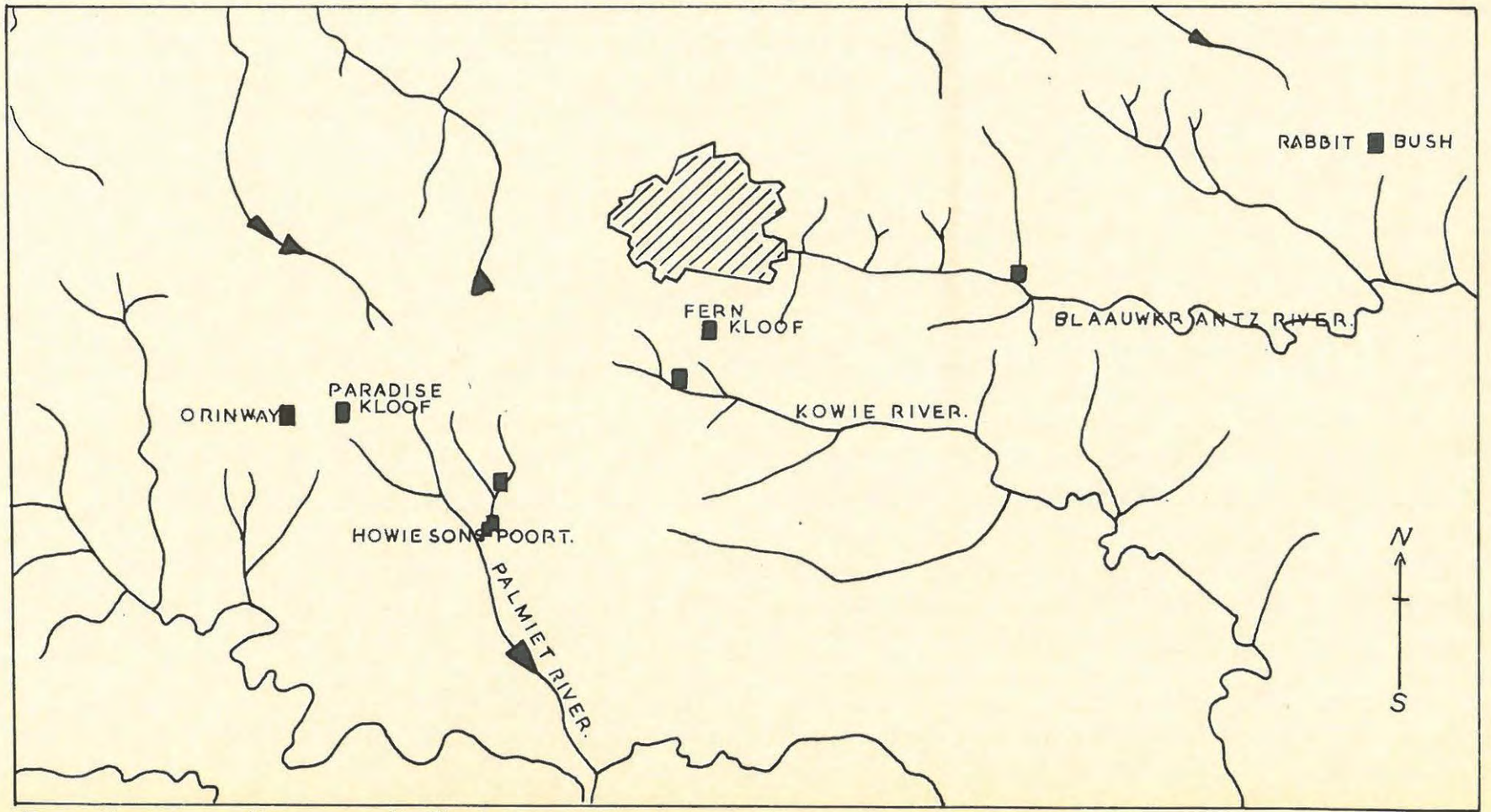
1. METHOD OF COLLECTION AND EXAMINATION.

Decaying plant material was collected in jars or plastic bags. Initially, the decaying plant material, chiefly leaf skeletons, was placed in shallow dishes of stream water and under these conditions the fungi ceased to produce conidia after about two days. It was found that, if the leaves were placed in jars of water which was constantly aerated, it was possible to prolong spore production for about a week, except in the very hot weather, when there was a rapid multiplication of bacteria even in the aerated water. In very hot weather the jars were placed in the refrigerator in an attempt to control the multiplication of bacteria.

The spore development of the fungi growing on the decaying leaves was followed by dissecting out suitable pieces of leaf and suspending these in hanging drop cultures. At first closed rings were used but in several cases, under these conditions certain irregularities in the shape of the spores developed were observed. It was decided that this might be due to the development of anaerobic conditions, and so rings with an inlet and outlet tube were used and air was passed through the ring for a few minutes approximately once an hour (see Fig.1). Under these conditions spore development appeared to be quite normal. The drawings of the developing spores and of other morphological features of these fungi were all made with the aid of a camera lucida.

2. DESCRIPTION OF SOURCES OF MATERIAL.

The aquatic Hyphomycetes described here were collected from the following four main areas of the Eastern Cape Province of South Africa:- (1) the Grahamstown district, (2) Port Elizabeth, (3) the Hogsback in the Amatola Mountain Range and (4) from near Alice (see map 1). The sites within these main localities are as follows:-



■=collecting site.
▶= dam.
///=Grahamstown.

SCALE 1inch=2 miles.

MAP 2.

MAP OF GRAHAMSTOWN AREA.

1. Grahamstown district (Map 2).

(a) A streamlet in an islet of natural forest in Fern Kloof on the south facing slope of the hills south of Grahamstown. The collection site was a few feet from the spring head at which point the streamlet is a shallow trickle.

(b) A stream, the headwaters of the Kowie River, in Featherstone Kloof below Fern Kloof. The stream at this site is overhung by oak trees, Quercus robur.

(c) The Blaauwkrantz River, five miles from Grahamstown on the Belmont Valley road. The stream is overhung by trees of Populus nigra and Quercus robur. Decaying leaves of these trees are held back by rocks in the shallows, where the water is running swiftly.

(d) A small stream in an islet of natural forest in Paradise Kloof on a south facing slope six miles from Grahamstown on the Highlands road. The stream is shallow fast flowing and at intervals cascades over rocks in the stream bed.

(e) A stream running in a south westerly direction on the farm, Orinway, seven miles from Grahamstown on the Highlands road. In its higher reaches the stream passes through fynbos but at the collection site it is overhung by trees of Quercus robur and Populus nigra. It is fast flowing and the decaying leaves are held back by stones in the shallows.

(f) Two tributaries of the Palmiet river in Howiesons Poort on the Grahamstown - Port Elizabeth road. One of these streams is overhung by indigenous trees and shrubs and by the exotic shrub Acacia longifolia. The other stream is overhung by trees of Populus sp., Quercus robur and by a few indigenous trees such as Harpephyllum caffrum.

(g) A stream, a tributary of the Blaauwkrantz river in a large islet of natural forest adjoining the Rabbit Bush on a south facing hill slope about ten miles from Grahamstown on the East London road.

2. Port Elizabeth, the Baakens river, Settler's Park.

(a) In a plantation of Populus sp. The fallen leaves are caught on sticks lying across the stream.

(b) Rocky shallows beneath trees of Salix babylonica below a weir.

3. The Hogsback, Amatola Mountain Range

(a) Seven streams in natural forest on south facing slopes, four in the lower forest and three in the upper forest.

(b) A streamlet running through a plantation of Quercus robur.

(c) Two streamlets running through plantations of Populus serotina (? P. nigra x P. deltoides). In one stream the fallen leaves lie on the surface of the mud and a trickle of water runs over them, while in the other stream the fallen leaves are caught on sticks which lie across the bed of the stream near the surface of the water.

(d) Two streams in mixed plantations of exotic trees. The fallen leaves are caught on sticks lying across the stream bed near the surface.

(e) Two streams in natural forest on the south facing slopes of Wolf Ridge.

(f) Two streams running through fynbos on a south facing slope above the forest line near the Kettle Spout Falls.

4. Near Alice - the Tyumie River, eight miles from Alice on the road to the Hogsback.

Decaying leaves of Combretum caffrum and Salix babylonica are held up behind stones in the shallows, where the water is running swiftly.

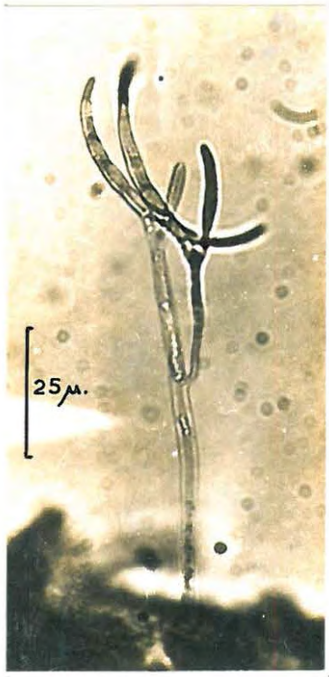
One scant collection of decaying leaves and water was also made from the Blaauwkrantz River, where it passes through natural forest in the Blaauwkrantz Pass in the Tzitzikamma Mountains.

3. DESCRIPTION OF SPECIES AND OF SPORE DEVELOPMENT.

Two types of conidia are found in the Hyphomycetes. These are known as terminal thallospores or aleuriospores and phialospores. The definitions given by Ingold (1957) are quoted below:

> "The terminal thallospore represents the swollen end of a hypha which is cut off at an early stage by a cross wall as a spore primordium. After further development the spore separates from its conidiophore, usually by disarticulation at the junction of the two structures, although sometimes liberation is achieved by breakdown of a separating cell at the end of the conidiophore. Although the vacant conidiophore may grow and produce another spore, it does not usually do so, and if it does the new spore is not normally formed exactly at the same level as the first".
>
> "The phialospore is produced from a special organ, the phialide, occurring at the end of the conidiophore. The phialide is typically wider in the middle tapering to a narrow neck at its apex. From the

PLATE I.



Lunulospora curvula Ingold. Conidiophore with two branches each bearing at its tip two young conidia, one almost mature and the other very young. Growing on a decaying leaf of Populus nigra from the Belmont Valley. Mounted in acid fuchsin and lactophenol.

end a spore is produced and this grows to full size before being cut off by a wall. Immediately one phialospore is formed another begins to develop from the phialide succeeding phialospores being produced at exactly the same level".

Of the eighteen species found all except two could be assigned to described species. The other two are new species of Articulospora and Anguillospora and are described in this thesis. All the descriptions of genera and known species are, unless otherwise stated, taken from Professor Ingold's publications. Three spore types, an "Articulospora" type, an "Anguillospora" type and a Y-shaped spore were also found. However the material collected was insufficient for an identification of these spores to be made.

Lunulospora Ingold.

"Submerged aquatic fungi with branched, septate mycelium. Conidiophore simple or branched. Conidium (aleuriospore) unicellular, sigmoid or crescent-shaped, borne terminally on the conidiophore on a small stalk cell attached not to an end of the spore but at a point some way from either end. Spore liberated by the breakdown of the stalk cell". (Ingold 1942).

Lunulospora curvula Ingold (Pl. I).

"Submerged aquatic fungus with branched septate mycelium. Conidiophore simple or sparingly branched, 50–200 mu. long, 2–2.5 mu. broad. Conidium (aleuriospore) produced terminally on a short stalk cell 3–5 mu. long 1.5 mu. broad. By repeated budding from the end of the conidiophore a number of spores may be produced in succession (but not basipetally),

each seated on a stalk cell. Aleuriospore unicellular, crescent-shaped or sigmoid, 70-90 mu. long, 4-5 mu. broad in its middle region but tapering to 1.5 mu. at its ends, with a row of conspicuous vacuoles. Attached at a point along its convex surface to the stalk cell. Liberated spore, with an inconspicuous hilum where it was originally attached to the stalk cell.

On submerged decaying leaves of Alnus glutinosa and Salix sp. from a stream in Leicestershire, England". (Ingold 1942).

Other records of Lunulospora curvula are from California (Ranzoni 1953), from Jamaica (Hudson and Ingold 1960), from Sweden (Nilsson 1958 and Willen 1958) and from Rhodesia and Uganda (Ingold 1958). This fungus was found on submerged decaying leaves of the exotic trees, Populus serotina, Quercus robur and Eucalyptus globulus: of the indigenous trees Celtis africana, Xymalos monospora and Maytenus cymosus: of Rubus sp: and on a decaying fruit of Acacia sp. from the Hogsback, on decaying leaves of the exotic trees, Populus nigra from the Belmont Valley and Quercus robur from Featherstone Kloof, and of the indigenous trees, Rhus legati, Rapanea melanophloeos, Ficus capensis and Harpephyllum caffrum from Howiesons Poort: Ocotea bullata, Cassine sp. and Celtis africana from Paradise Kloof: Rhus legati, Celtis africana and Cunnonia capensis from the Rabbit Bush and Combretum caffrum from the Tyumie river: and of the exotic tree Acacia sp. from the Rabbit Bush.

The conidiophores are produced most abundantly from the mid-rib and lateral veins of the leaves rather than from the margins or petioles. They were most abundant in collections made in autumn, April and May, but showed a marked decrease in abundance in the winter months,

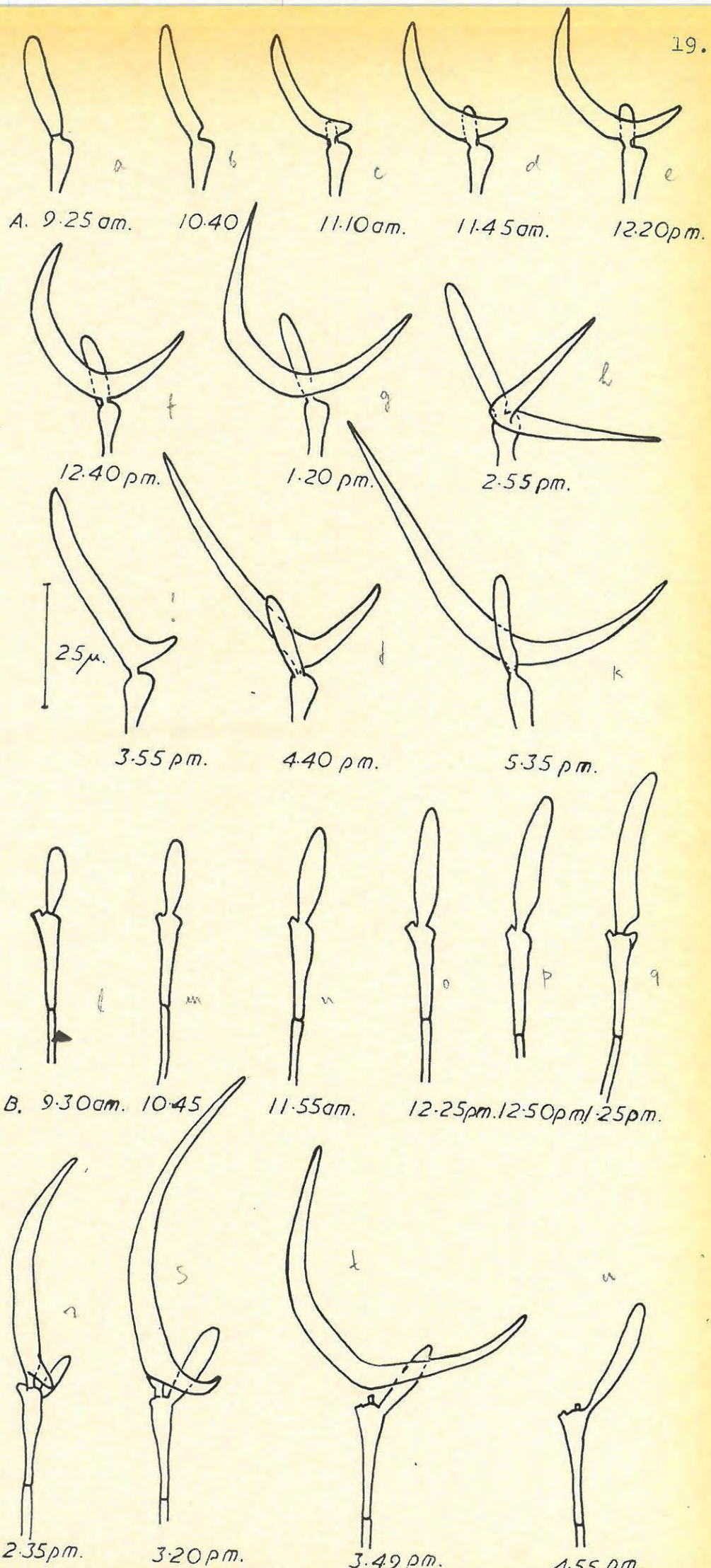
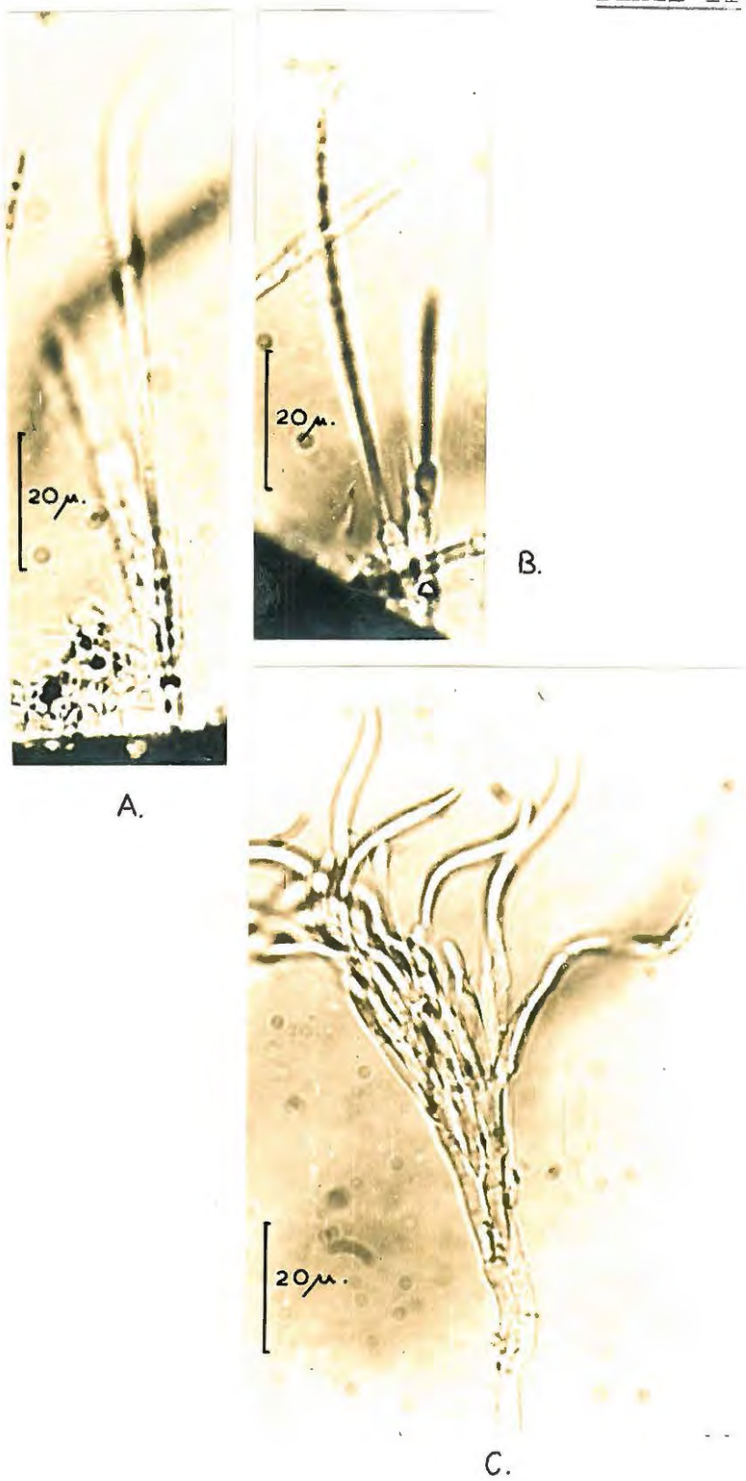


Fig.2. *Lunulospora curvula* Ingold. Spore development. From a hanging-drop culture of the fungus growing on a decaying leaf of

June, July and August, remaining scarce until the following April except for a slight peak in November. It was observed that there was a considerable variation in the length of the conidiophore projecting from the leaf tissue. The length was regularly about 13 mu. in the fungus growing on oak leaves from Featherstone Kloof, whereas it was 50-80 mu. in the fungus growing on poplar leaves from the Belmont Valley.

Spore development was followed in hanging drop cultures of the fungus growing on decaying leaves of Populus nigra and Xymalos davyi (Fig. 2). While the development of a spore is proceeding, the primordium of the succeeding spore arises as a protruberance on the end of the conidiophore to one side of the first spore. Ingold (1942) observed that the protruberance was first cut off from the conidiophore by a septum and that a second septum was formed cutting off a small basal cell which is the stalk cell. These two septa were not, however, always apparent (see second septum Fig. 2a, both septa Fig. 2,t). The spore primordium enlarges and elongates and after about three and a half hours a second growing point develops near its base (Fig. 2,h,i,p,q). Both growing points continue to grow until a crescent-shaped or sigmoid spore is developed (Fig. 2,g,h,t). The spore takes about six hours to develop at laboratory temperature as compared with ten hours observed by Ingold (1942). The spore is liberated by the breakdown of the stalk cell. Whereas Ingold (1942) observed that the remains of the stalk cell were never left attached to the spore or the conidiophore, they have often been found attached to the conidiophore but not to the spore (Fig. 2,t).

PLATE II.



A.&B. Flagellospora curvula Ingold. Two groups of conidiophores each consisting of a group of phialides. Spores on right young, that on left almost fully grown. Growing on a decaying leaf of Combretum caffrum from the Tyumie River. Mounted in water.

C. Flagellospora penicillioides Ingold. Conidiophore from culture of the fungus growing on two percent malt agar. Mounted in water.

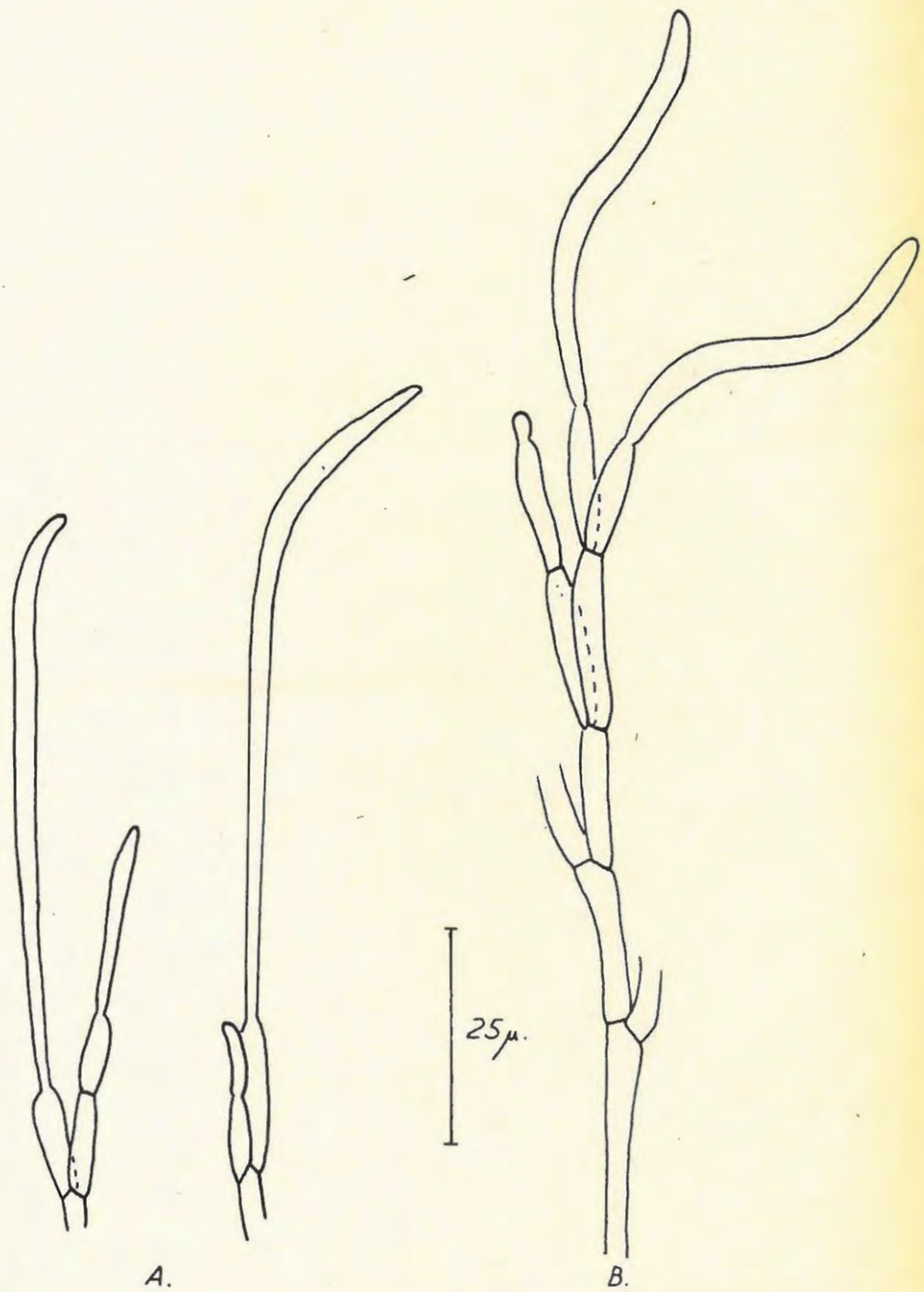


Fig.3. A. Flagellospora curvula Ingold. Two groups of conidiophores each consisting of a group of phialides. Two young spores and two almost mature. Growing on a decaying leaf of Combretum caffrum from the Tyumie River.
B. F. penicillioides Ingold. Conidiophore from culture of the fungus on two percet malt agar.
A and B mounted in water.

Flagellospora Ingold.

"Submerged aquatic fungi with branched septate mycelium. Conidiophore branched to form a number of phialides. Conidia (phialospores) hyaline, filiform, produced in basipetal succession". (Ingold 1942).

Flagellospora curvula Ingold.

(Pl. II A & B) (Fig. 3).

"Submerged aquatic fungus with branched septate mycelium. Conidiophore usually branched forming a group of two to ten phialides. Each phialide clavate, 10-20 mu. long, 2.5 mu. broad, producing conidia (phialospores) in basipetal succession. Conidium curved or sigmoid, hyaline unicellular, 100-150 mu. long, 2 mu. broad in the middle region tapering to 1.5 mu. towards its ends.

On submerged decaying leaves of Alnus glutinosa and Salix sp. from a stream in Leicestershire, England". (Ingold 1942).

Other records of Flagellospora curvula are from California (Ranzoni 1953), from Switzerland (Ingold 1949), from Sweden (Nilsson 1958), from Ghana (Dixon 1959) and from Rhodesia and Uganda (Ingold 1958). This fungus has been found growing on submerged decaying leaves of the exotic trees, Populus serotina and Quercus robur: of the indigenous trees, Xymalos monospora, Rhus legati, Fagara davyi and Podocarpus latifolius and of Rubus sp. from the Hogsback and on skeletonised leaves of the indigenous tree, Combretum caffrum, from the Tyumie River. The conidiophores of this fungus are found with equal frequency on the veins and margins of the decaying leaves. Flagellospora curvula was common in August but not during the rest of the year.

Flagellospora penicillioides Ingold.
(Pl. II C) (Fig. 3).

"Submerged aquatic fungus with branched, septate mycelium on malt agar hyaline at first, later bright brown with moniliform chains of inflated, oil containing brown cells (chlamydospores) 15–35 mu. long, 8–16 mu. broad, occurring in the older parts of the aerial mycelium. Aquatic conidiophores hyaline, branched as in Penicillium: unbranched stalk part 150-300 mu. long, 2.5 mu. broad, apical phialides 8–40, 15–20 mu. long, 2.5 mu. broad. Conidia (phialospores) hyaline, 45–55 mu. long, 2.5 mu. broad, in the middle but tapering to 1.5 mu. at the ends, unicellular or uniseptate, curved or more usually slightly sigmoid, produced in basipetal succession from the phialides. Aerial conidiophores produced sparingly on malt agar, resemble the aquatic conidiophores but produce heads of slimy conidia.

On submerged decaying leaf from a stream near Reading, England". (Ingold 1944).

Flagellospora penicillioides has also been recorded from California (Ranzoni 1953) and from Jamaica (Hudson and Ingold 1960). This fungus was found in August growing on a submerged decaying leaf of Maytenus cymosus from the Hogsback and has been isolated in culture on malt agar from spores from the Belmont Valley. The cultural characteristics of this fungus agree with the description quoted above. However, spore production from strips of the culture placed in sterile water took three to four days to commence, whereas Ingold (1944) found that spores were produced within two days.

PLATE III.

A.

B.

A. Anguillospora longissima (Sacc. & Syd.) Ingold. Conidiophore with conidium. Growing on a decaying leaf of Populus sp. from the Baakens River. Mounted in water.

A. Anguillospora filiformis n.sp. Showing point of attachment of conidium to conidiophore and process at base of conidium. Growing on a decaying leaf from the Hogsback. Mounted in water.

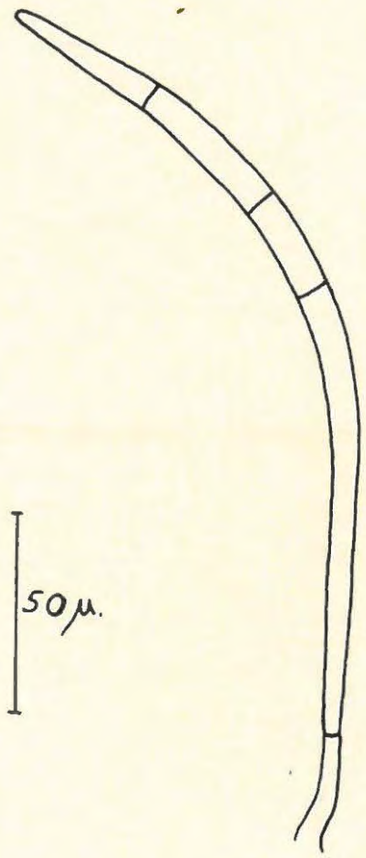


Fig. 4. Anguillospora longissima (Sacc. & Syd.) Ingold. Conidiophore bearing a conidium. Growing from a decaying leaf of Populus sp. from the Baakens River. Mounted in water.

Anguillospora Ingold.

"Submerged aquatic fungus with branched, septate mycelium. Aleuriospores terminal, eel-like, septate, colourless, either separating from the aleuriophore by the breakdown of a separating cell or by a disarticulation and rounding off process at a septum at the end of an aleuriophore". (Ranzoni 1953).

Anguillospora longissima (Sacc. & Syd) Ingold.
(Pl. III A)(Fig. 4).

"Submerged aquatic fungus with branched, septate mycelium which is hyaline when young, becoming greenish grey when old. Conidiophore usually simple, 50-150 mu. long, 2-4 mu. broad. Conidium (aleuriospore) terminal, 200-350 mu. long, 5-6 mu. broad in middle region tapering to 3-4 mu. broad at ends, 6-10 septate, curved or sigmoid, separating when mature by the breakdown of a "small separating cell" at the end of the conidiophore. On submerged decaying alder and willow leaves, England". (Ingold 1942).

Other records of this fungus are from California (Ranzoni 1953), from Sweden (Nilsson 1958 and Willen 1958), from Japan (Tubaki 1957), from Jamaica (Hudson and Ingold 1960) and from Uganda (Ingold 1958). Anguillospora longissima has been found growing on submerged leaf skeletons of the indigenous tree, Maytenus cymosus: of Rubus sp. from the Hogsback and of the exotic trees, Populus serotina from the Hogsback: Populus nigra from the Belmont Valley and Populus sp. from the Baakens River. It was found in collections made in April, May, July and August and was absent at

other times of the year. The conidiophores are more commonly produced from the mid-rib, petiole and lateral veins of the leaf than from the margin. The spores germinated on two per cent malt agar but attempts to obtain Anguillospora longissima in culture were unsuccessful.

Anguillospora filiformis n. sp.
(Pl. III B).

Submerged aquatic fungus, mycelium embedded in tissue of decaying leaf. Conidiophore simple, 3 mu. broad, slightly inflated towards the apex. Conidia (aleuriospores) terminal, elongate, septate, hyaline, 200-250 mu. long, 3.8 mu. broad. Sides of spore almost parallel converging towards the apex. Small lateral outgrowth on side of spore near base, becoming more or less parallel with sides of spore.
Hab. On submerged decaying leaves from streams in the Eastern Cape Province of South Africa.

Anguillospora filiformis sp. nov.

Fungus aquaticus, submersus, mycelio in folio putrescenti inserto. Conidiophora simplicia 3 mu. lata, subinde apice moderate inflata. Conidia (aleuriosporae) acrogena, elongata, septata, hyalina, 200-250 mu. longa, 3.8 mu. lata. Latera spori paene parallela, ad apicum alternata. Parvus processus lateralis prope ad basim spori situs, lateribus spori plerumque parallelus.
Hab. In folius putrescentibus, in fluminibus in orientale parte Capicae Provinciae Africae Australis submersis.

This species was found growing on submerged decaying leaves of the exotic trees, Populus serotina, Quercus robur and Eucalyptus globulus: of the indigenous trees Maytenus cymosus, Fagara davyi and Royena lucida from the Hogsback, Rapanea melanophloeos from Paradise Kloof, Rhus legati from Paradise Kloof and Fern Kloof and Cunnonia capensis from Rabbit Bush. Spores of Anguillospora filiformis were common in the streams of Paradise Kloof and Howiesons Poort from June to August but conidiophores were never found in abundance.

The conidium of Anguillospora filiformis differs from those of Anguillospora longissima, A. crassa Ingold, A. pseudolongissima Ranzoni and A. gigantea Ranzoni in that it is only 3.8 mu. broad, whereas the conidia of the latter four species are 5.5 mu. 15-20 mu., 4.6-6 mu. and 5-6 mu. broad respectively. It further differs from those of the other four species in the presence of a lateral process at the base, an appendage which they lack. The sides of the conidium of this fungus are parallel for most of their length but in A. flagellifera Ingold, in which a basal process is also produced, the conidium consists of two unlike portions, a truncate basal portion 10-14 mu. broad and an upper whip-like portion (see Table 3).

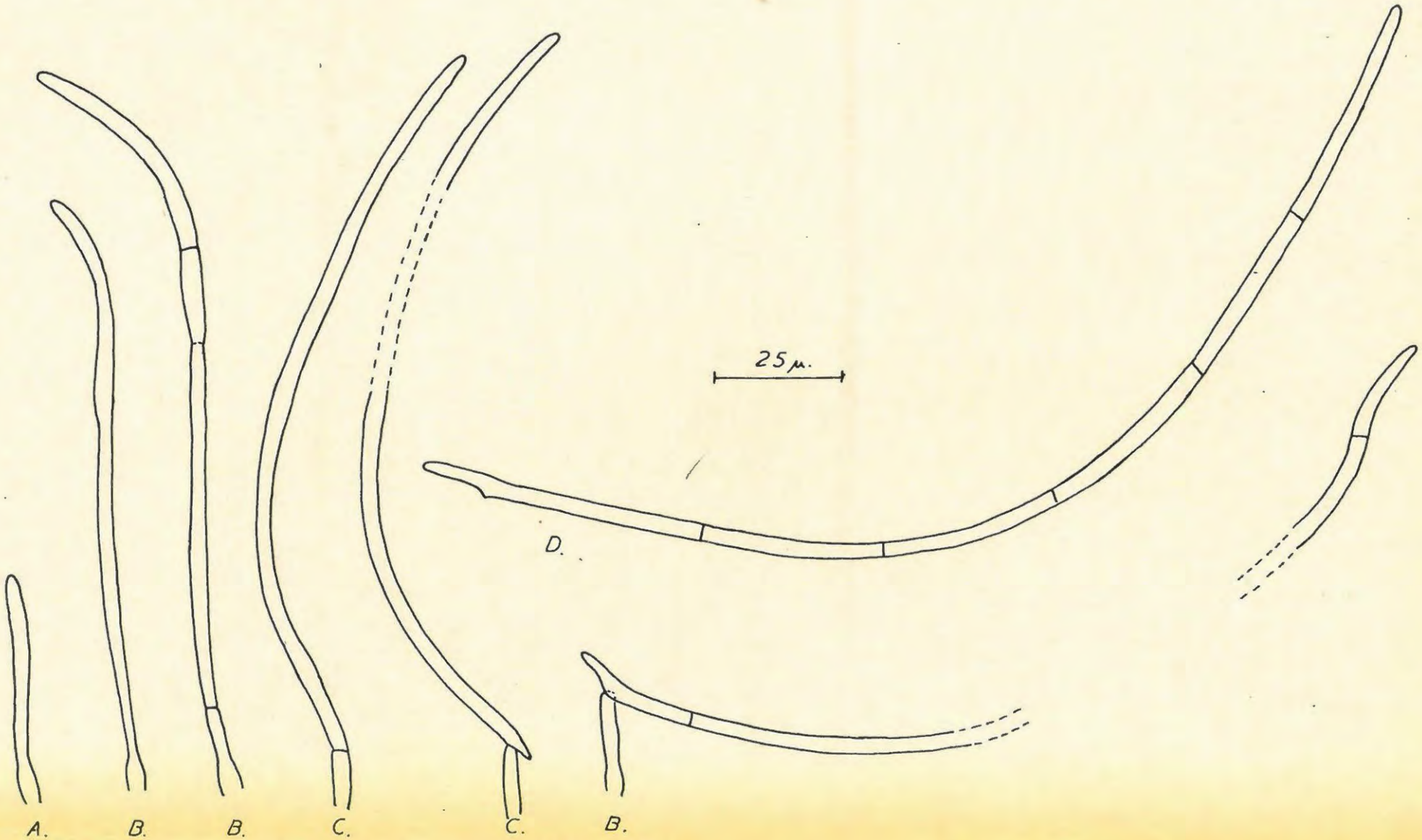


Fig.5. Anguillospora filiformis. n.sp. Spore development. From hanging-drop cultures of the fungus growing on decaying leaves of Rapanea melanophloeos.

Table 3.

TABLE OF COMPARISON OF THE ANGUILLOSPORA SPP.

Anguillospora spp.	Length in mu.	Width in mu.			
		Base	Middle	Apex	Basal Process
A. longissima	200-350	3-4	5-6	3-4	lacking
A. crassa	120-200	8-10	15-20	8-10	"
A. pseudolong-issima	50-100	6-8	4.6-6	3-5	"
A. gigantea	150-750	3-5	5-6	2.5-3	"
A. flagellif-era	140-200	2.5-3	10-14	2-3	present
A. filiformis	200-250	3.8	3.8	3.8	"
A. sp. (Unidentified)	128	4.5	6	1.5	"

Observations of spore development in Anguillospora filiformis have been made from hanging drop cultures of the fungus growing on leaf skeletons of Rapanea melanophloeos. A complete series of drawings of spore development of one spore has not yet been made but drawings of these spores (Fig. 5,A,B and C) at various stages in their development have been arranged in a developmental series. The spore primordium (Fig. 5,a) which is somewhat narrower than the inflated portion of the conidiophore elongates and becomes slightly broader in the upper half (Fig. 5,b). However, as the spore continues to elongate the sides become more or less parallel converging towards the apex (Fig. 5,d). A small knob-like process appears on the side of the spore immediately above the septum which has formed between the developing spore and conidiophore (Fig. 5,e). This process elongates to form a short peg-like process which is about half as broad as the spore and which is orientated with its long axis almost parallel with the sides of the spore (Fig. 5,g). During its development the

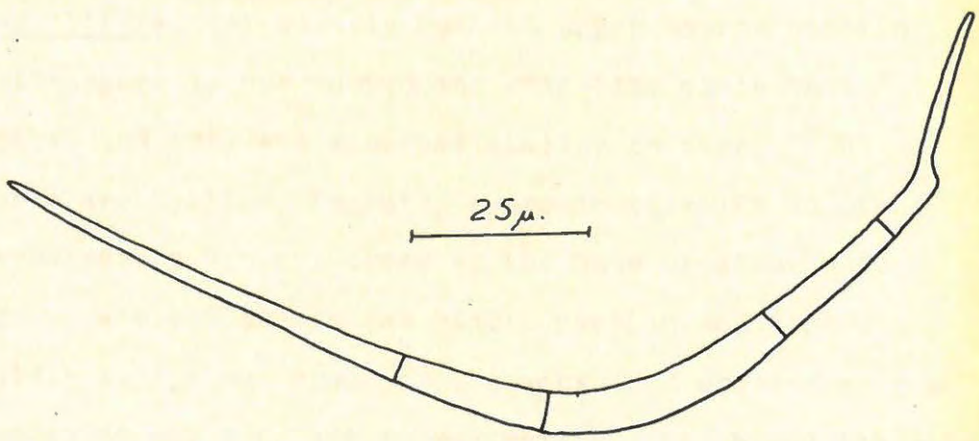


Fig. 6.
Anguillospora sp. Free floating spore from Poplar plantation Hogsback. Mounted in water.

spore becomes septate. Liberation of the spore seems to be by disintegration of the middle lamella of the septum between it and the conidiophore. It would appear that in reaching its full size and ultimate angle, the process may assist in "pushing" the spore off the conidiophore. The spores germinate readily on two per cent malt agar but the fungus has not as yet been obtained in culture.

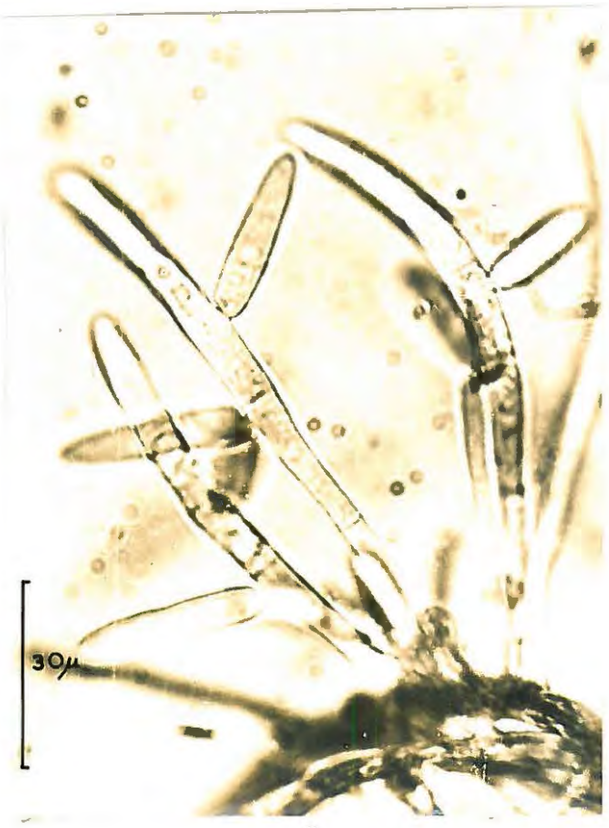
Anguillospora sp. (Fig. 6).

Spores, probably of a species of Anguillospora, were found in stream water from the upper natural forest at the Hogsback in April 1961 (Fig. 6). The spores do not correspond exactly with the descriptions of the named species. They resemble spores of Anguillospora flagellifera most closely but the upper narrow portion of the spore is not curved and whip-like as in that species and they are somewhat smaller in size. The spores are hyaline, septate, approximately 128 mu. long, approximately 4.5 mu. broad at the base broadening to approximately 6 mu. in the middle portion and tapering rapidly to 1.5 mu. broad at the apex. A process approximately 23 mu. long and approximately 3 mu. broad tapering to 1 mu. broad arises from the base of the basal cell at an angle to the sides of the spore. By considering the table of comparison (Table 3) of the species of Anguillospora it will be seen that these measurements do not correspond with those of any of the described species.

PLATE IV.

A.

B.



C.

Tricladium splendens Ingold.

A.&B. Growing on a decaying leaf of Populus serotina from the Hogsback. Mounted in water.

A. Group of conidiophores bearing conidia in various stages of development.

B. Conidiophore bearing a young conidium.

C. Conidiophores bearing young conidia. From a culture of the fungus growing on malt agar. Mounted in water.

Tricladium Ingold.

"Submerged aquatic fungi with branched, septate, mycelium. Conidium (aleuriospore), septate hyaline, consisting of an elongated main axis with two lateral branches arising at different levels from the main axis". (Ingold 1942).

Tricladium splendens Ingold.
(Pl. 1V A, B and C).

"Submerged aquatic fungus with branched, septate, mycelium. Conidiophore usually simple. Conidium (aleuriospore) terminal, hyaline, consisting of a main axis, fusiform, 3-6 septate 60-20 mu. long, 6-7 mu. broad at widest part, tapering to 2-3 mu. at their apices, with a narrow isthmus, 2 mu. wide, where each branch joins the main axis of the spore. The two branches originate from the main axis of the spore at levels 10-20 mu. apart.

On submerged decaying leaves of Alnus glutinosa from a stream in Leicestershire, England". (Ingold 1942).

Tricladium splendens has also been recorded from California (Ranzoni 1953), from Switzerland (Ingold 1949), from Sweden (Nilsson 1958) and from Japan (Tubaki 1957). This fungus has been found growing on submerged decaying leaves of the exotic trees Populus serotina and Quercus robur: of the indigenous tree, Maytenus cymosus and of Rubus sp. from the Hogsback. Conidiophores were common in collections made in July and August but absent from all other collections except in April 1961 in which it was rare. The conidiophores are produced more commonly from the mid-rib, lateral veins and petioles of the decaying leaf than from the margins.

Spore development has been followed in a hanging drop culture of Tricladium splendens growing on a decaying

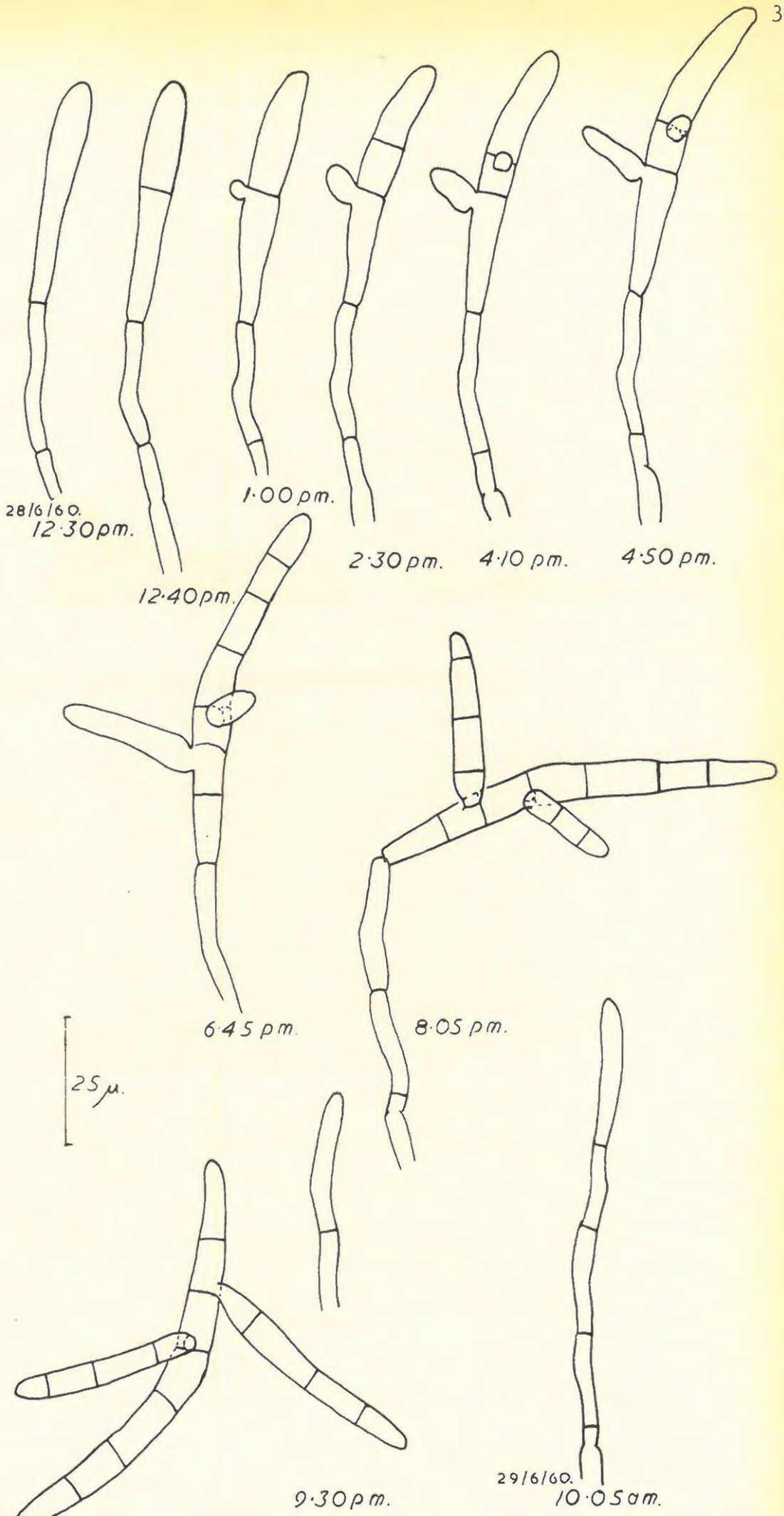
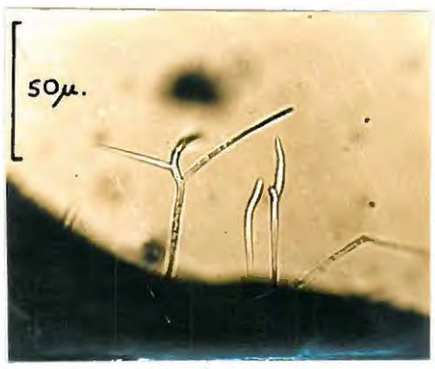
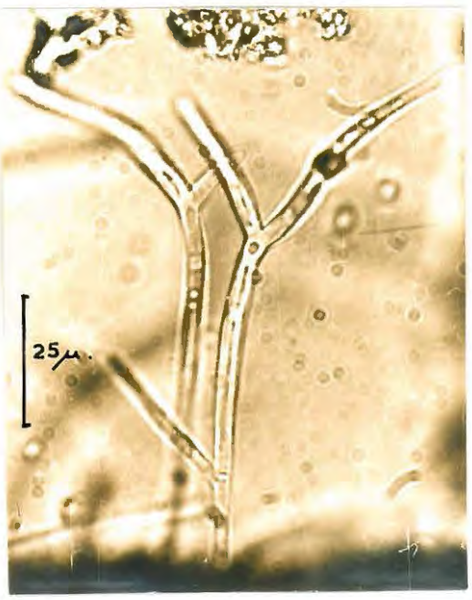


Fig. 7 *Tricladium splendens* Ingold. Spore development. From a hanging-drop culture of the fungus growing on a a decaying leaf of *Populus serotina*.

PLATE V.



A.



B.

C.

Tricladium gracile Ingold.

A.&B. Growing on a decaying leaf of Quercus robur from the Hogsback. Mounted in water.

A. A group of conidiophores bearing conidia in various stages of development.

B. Two conidiophores each bearing an imature conidium.

C. A slice from a culture of the fungus growing on malt agar and submerged in aerated water. Conidiophores bearing conidia in various stages of development. Mounted in water.

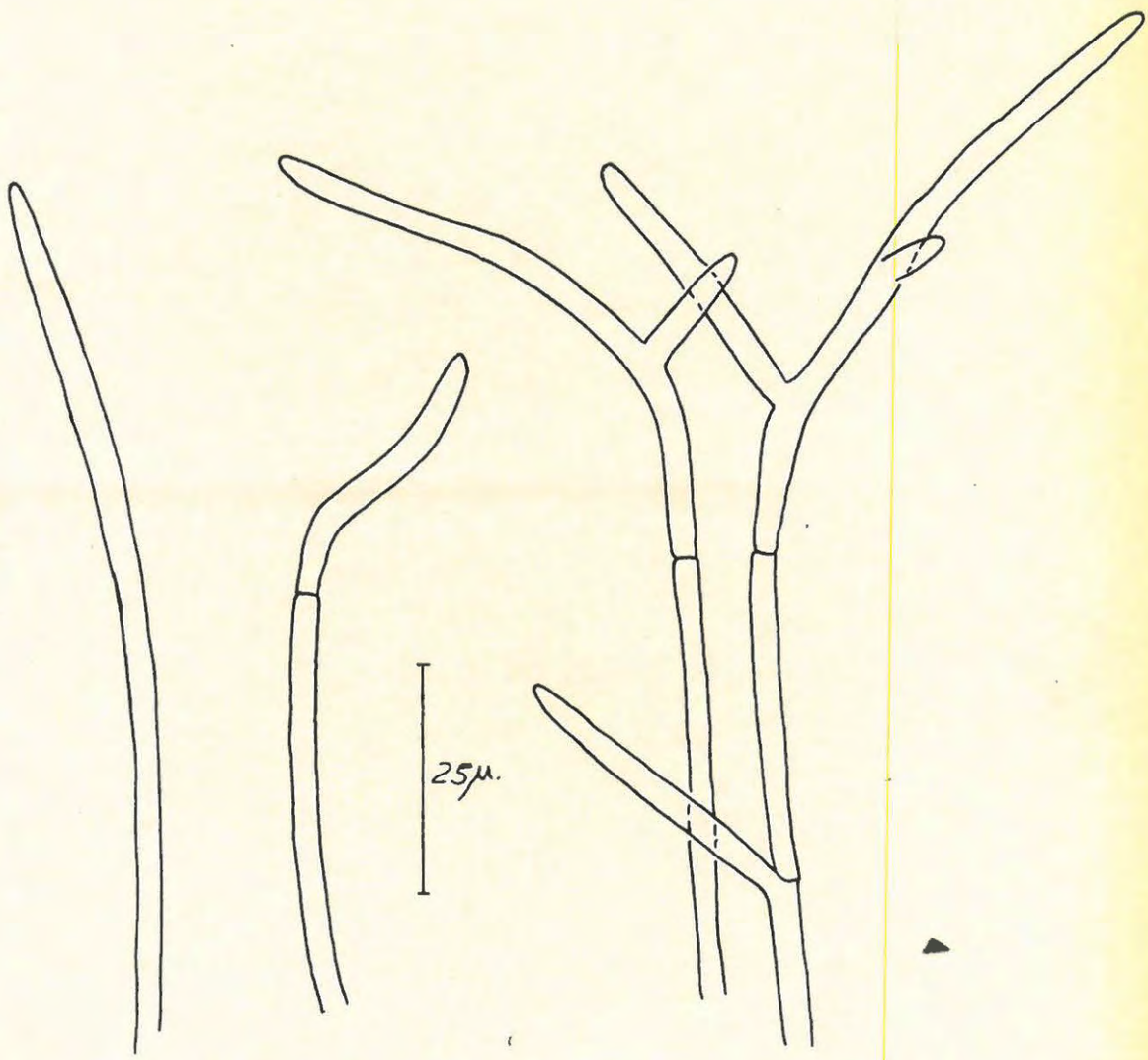


Fig.8. *Tricladium gracile* Ingold. Conidiophores bearing imature conidia. Growing from decaying leaf of *Quercus robur* from the Hogsback. Mounted in water.

leaf of Populus serotina (Fig. 7).

The spore primordium develops as a club-shaped swelling at the end of a thin straight conidiophore from which it is cut off by a cross wall (Fig. 7,a). As the primordium elongates it becomes slightly curved and a septum is formed about half way along its length (Fig. 7,b). A protruberance, which will form one of the lateral arms, arises just below this septum and is constricted at its base (Fig. 7,c). A second septum forms a short distance above the first (Fig. 7,d) and a protruberance, which will form the second lateral arm, arises just below this septum and is constricted at its base (Fig. 7,e). The two lateral arms are never in the same plane. The main axis of the spore and the two laterals elongate and become further septate (Fig. 7,h). The spore is liberated by disjunction at the cross-wall separating it from the condiiophore. This pattern of development confirms that of Ingold (1942). After the first spore has been liberated the conidiophore elongates and a few cells are added to its length before another spore primordium is cut off in the same way as the first (Fig. 7,j). Spore development from the formation of the primordium to the liberation of the spore takes about eight hours.

The spores germinate on two per cent malt agar and the fungus has been isolated in pure culture on this medium. The cultural characteristics are given in Part 3. Conidia were produced when strips of culture were transferred to sterile stream water which was constantly aerated (Pl. 1V, B).

Tricladium gracile Ingold.

(Pl. V,A,B and C)(Fig. 8).

"Submerged aquatic fungus with branched, septate mycelium. Conidiophore simple, hyaline, 10–50 mu. long, 2–2.5 mu. broad. Conidia (aleurio-

spores) terminal, solitary, hyaline, septate, branched, consisting of a main axis and two branches, main axis 90–150 mu. long, 3.0–3.5 mu. broad in basal half, but tapering to 1.5 mu. towards the apex curved or bent to an obtuse angle at the point of origin of each lateral branch: branches straight, 50–80 mu. long, 1.5 mu. broad: distance between points of origin of the two branches 14–18 mu. From submerged decaying leaves of oak from streams in Britain". (Ingold 1944).

Tricladium gracile has also been recorded from California (Ranzoni 1953). This fungus has been found on submerged leaf skeletons of the exotic trees, Populus serotina, Quercus robur and Eucalyptus globulus from the Hogsback and Populus nigra from the Belmont Valley: of the indigenous trees and shrubs, Celtis africana, Rhus legati, Grewia occidentalis, Maytenus cymosus, Podocarpus latifolius, Buddleja salicifolia, Royena lucida and Cassine sp. and of Rubus sp. from the Hogsback. The conidiophores of this fungus are produced with equal frequency on the mid-rib, lateral veins and margins of the inhabited leaf and were abundant from April to August but rare or absent at other times of the year.

The spores germinate readily on two per cent malt agar and the fungus has been isolated in pure culture on this medium. The cultural characters are given in Part 3. When strips of culture were transferred to sterile stream water which was constantly aerated, spores were produced. The conidiophores and conidia which arose from the dark cells of the old mycelium were colourless and agreed exactly with those formed in nature (Pl. V,C).

PLATE VI.

A.

B.

C.

Clavariopsis aquatica De Wild.

A.&B. Two conidiophores each bearing a conidium.
"A" before lateral branches have formed.
"B" almost mature.
Growing on a decaying leaf from the Hogsback.
Mounted in water.

C. Conidiophore bearing conidia in various stages of development. From a culture of the fungus growing on two percent malt agar and submerged in aerated water. Mounted in water.

Clavariopsis De Wild.

The following is a translation from the latin description by De Wildeman (1895, cited in Saccardo, 1913):

> Mycelium embedded, with simple erect free branches, septate, sending forth club-shaped branches at apex: the uppermost cells of the branch extended into three uni or bicellular branches. By the testimony of Lindau it is to be inquired whether the 1-3 cellular branches are to be considered as conidia.

Clavariopsis aquatica De Wild.

(Pl. VI).

> "Submerged aquatic fungus with branched, septate mycelium. Conidiophore usually simple, 50-250 mu. long, 2-2.5 mu. broad. Conidium (aleuriospore) terminal consisting of a broadly clavate, two-celled main part, 30-40 mu. long, 3-4 mu. broad at the base widening to 12-14 mu. broad at the apex, with three long divergent processes 50-70 mu. long, 1.5-2 mu. broad developed from its truncate apex". (Ingold 1942).

In addition to this record from England, Clavariopsis aquatica has been recorded from California (Ranzoni 1953), from Sweden (Nilsson 1958 and Willen 1958), from Japan (Tubaki 1957), from Jamaica (Hudson and Ingold 1960) and from Uganda (Ingold 1958). This fungus has been found growing on submerged leaf skeletons of the exotic trees, Populus serotina from the Hogsback, Quercus robur from the Hogsback and from the Belmont Valley and Populus nigra from the Belmont Valley: of the indigenous trees, Xymalos monospora and Maytenus cymosus, and of Rubus sp. from the Hogsback. Spores of this fungus have also been collected from the Rabbit Bush. The conidiophores

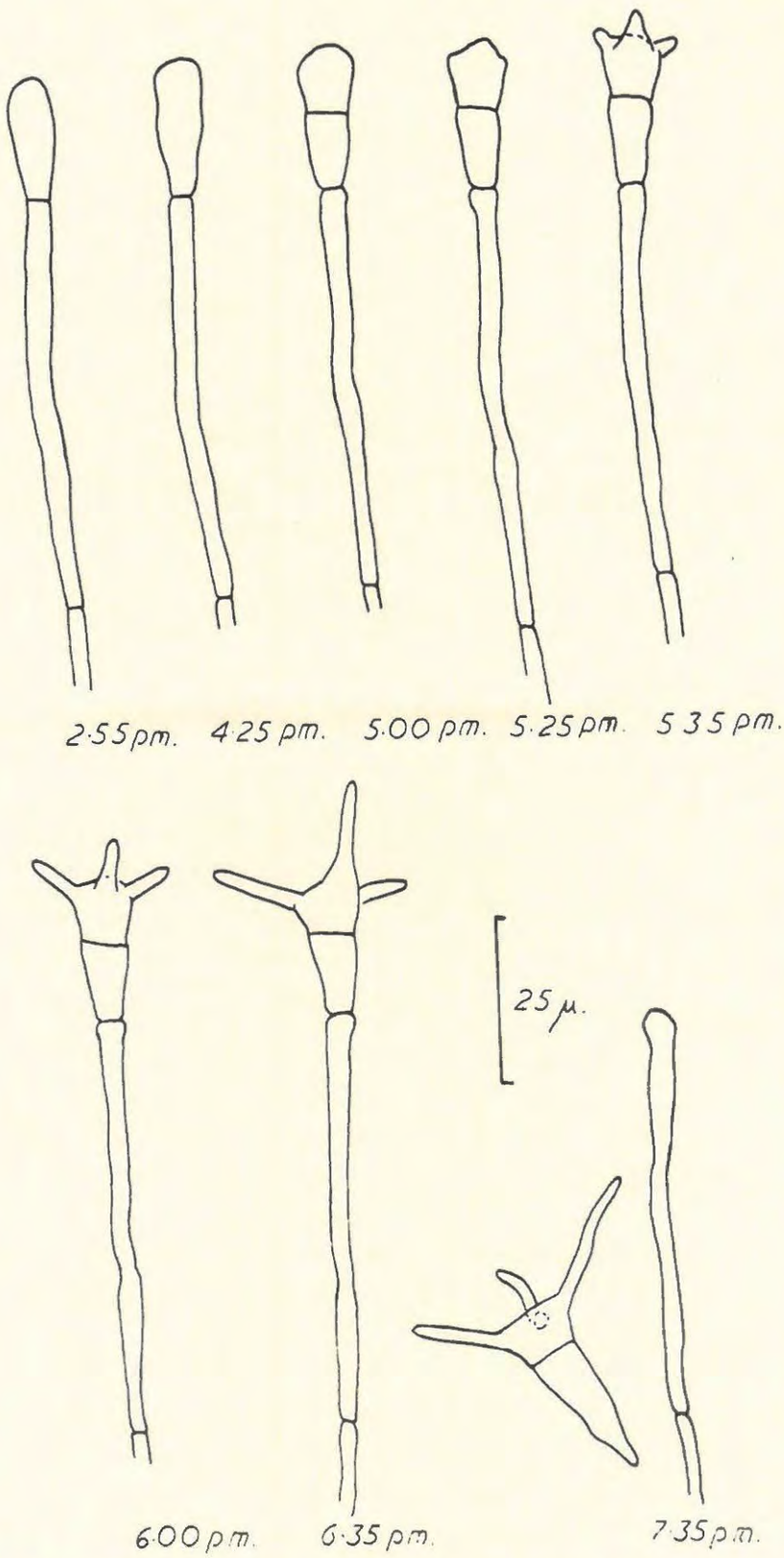


Fig. 9 Clavariopsis aquatica DeWild. Spore development. From a hanging-drop culture of the fungus growing on a decaying leaf of Populus nigra.

are produced more frequently from the mid-rib, lateral veins and petioles than from the margins of decaying leaves. Conidiophores were most abundant in the autumn months, March and April. They were also found in the winter and late summer but were not present in collections made during the spring and early summer, September to December. Ingold (1942) describes sclerotia and pycnidia produced by Clavariopsis aquatica growing on malt agar. Sclerotia only were produced by my isolate of this fungus. Pycnidia producing spores of the type described and illustrated by Ingold were, however, frequently found to be present on decaying leaves on which Clavariopsis aquatica was growing.

Spore development has been followed in hanging drop cultures of the fungus on decaying leaves of Populus nigra (Fig. 9). The tip of the conidiophore swells up and a cross wall is formed cutting off a clavate spore primordium (Fig. 9). A septum forms about half way along the length of the primordium dividing it into two cells (Fig. 9,a). Three bulges, which will form the three long divergent processes, appear at the distal end of the upper cell (Fig. 9,d). The spore is fully developed (Fig. 9,g) one to three hours later and is liberated by a rounding off of the two layers of the cross wall between the spore and the conidiophore (Fig. 9,h). This pattern of development closely resembles that described by Ingold (1942). It would appear that the formation of the cross-wall, which divides the spore into two cells, may take place either before or after the divergent arms have started to develop. In Ingold's drawings (1942) the wall is not present until after the divergent arms are quite well developed, whereas in this case in all the spores examined the wall was formed before the divergent processes started to develop. Some spores collected from the Belmont Valley had four

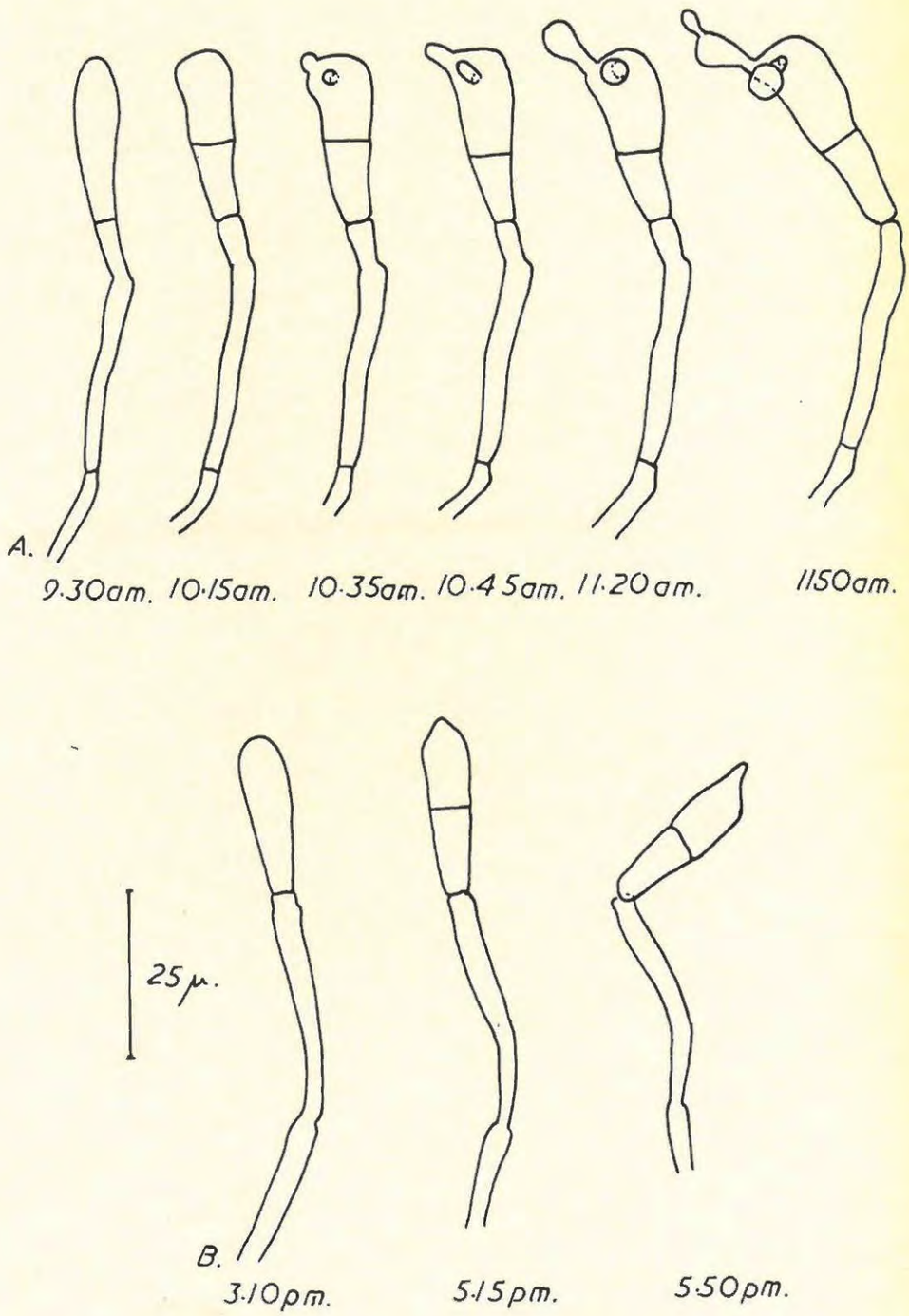


Fig.10. Clavariopsis aquatica De Wild. Abnormal spore development. From a hanging-drop culture of the fungus growing on a decaying leaf of Populus nigra.

divergent arms.

Two of the spores observed in hanging-drop culture, over a closed ring, developed abnormally. One of the spores formed was a simple two-celled spore with a small apical projection (Fig. 10,A) and the other had two short inflated arms, instead of the normal three long divergent arms, and one of these bore a secondary process (Fig. 10,B).

The spores germinate readily on two per cent malt agar made with stream water and pure cultures of the fungus have been isolated on this medium. The cultural characteristics are described in Part 3. When a strip of colony was transferred to water which was constantly aerated, conidiophores and conidia, which agreed exactly with those formed in nature, were produced from the dark cells of the old mycelium. (Pl. V1,C).

PLATE VII.

Heliscus longibrachiatus Ingold. Conidiophore bearing an almost mature conidium. Growing on a decaying leaf of Quercus robur from the Hogsback. Mounted in water.

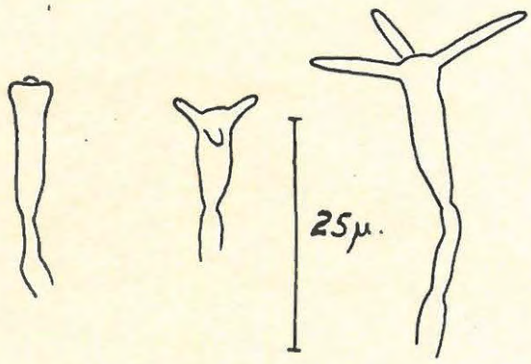


Fig. 11. Heliscus longibrachiatus Ingold. Conidia at various stages of development. Growing from a decaying leaf of Quercus robur from the Hogsback. Mounted in water.

Heliscus Sacc.

Translation of the latin description by Saccardo (1886):

> Sporodochium aplanate, slightly open (white). Conidia cylindrical, polygonal-headed at the apex in the form of a club, insignificant, supported by branched sporophores.

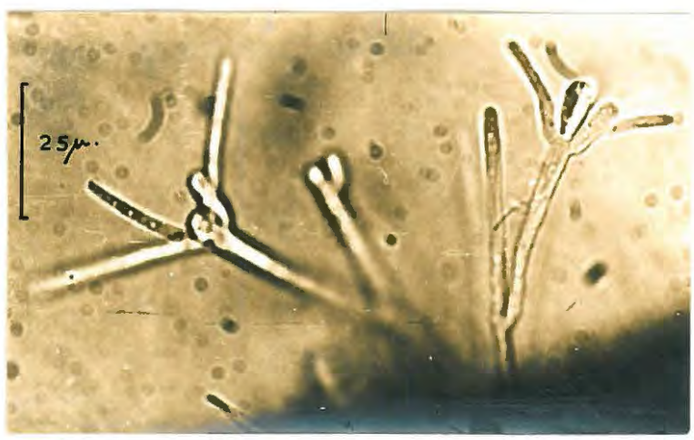
Heliscus longibrachiatus Ingold.

(Pl. VII)(Fig. 11).

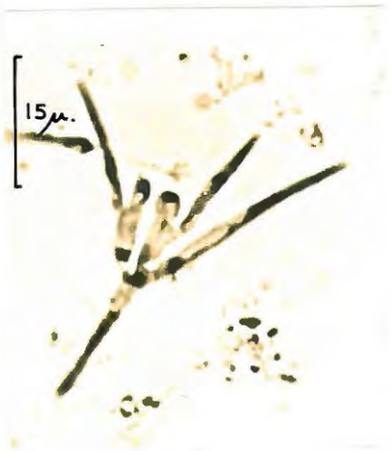
> "Aquatic fungus with branched septate mycelium. Conidiophores and conidia developed below water. Conidia usually simple consisting of a simple phialide 10-20 mu. long, 1.5 mu. broad at the base widening to 4.0 mu. at the apex, with three straight divergent arms 15-25 mu. long, 1.0-1.5 mu. broad arising from a truncate apex. Conidia unicellular produced in basipetal succession.
>
> On decaying leaves of Alnus glutinosa from a stream in Leicestershire, England". (Ingold 1942).

This fungus has also been recorded from Sweden (Nilsson 1958 and Willen 1958). Heliscus longibrachiatus has been found growing on submerged skeletonised leaves of the exotic trees, Populus serotina, Quercus robur and Eucalyptus globulus and on a decaying fruit of Acacia sp. from the Hogsback. This fungus was only abundant on leaves of Eucalyptus globulus and only in August.

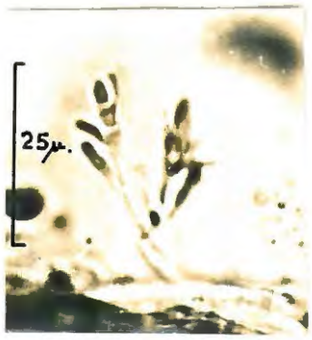
PLATE VIII.



A.



B.



C.

D.

E.

A. Tetracladium marchialanum De Wild. Two conidiophores each bearing two conidia in various stages of development. The conidium on the extreme left is almost mature. Growing on a decaying leaf of Combretum caffrum from the Tyumie River. Mounted in water.

B. Tetracladium marchialanum. Abnormal conidium from the Baakens River. Mounted in water.

C.,D.&E. Tetracladium setigerum (Grove) Ingold. Growing on a decaying leaf of Maytenus cymosus from the Hogsback.

C.&D. Branched conidiophores bearing conidia in various stages of development. Mounted in acid fuchsin and lactophenol.

E. Liberated conidium. Mounted in water.

Tetracladium De Wild.

The following is a translation from the latin by De Wildeman (1894, cited in Saccardo 1899):

> Saprophytic, emerging from and embedded in the plant tissue, multicellular: mycelium branched: fertile hyphae erect, branched with branches terminally, 2 or 3 small branches diverging, more or less sharply pointed at the ends: conidia arising from the wings of the small branches, oval, cylindrical or globose. Closest to the genus Titaeae.

Tetracladium marchialanum De Wild.

(Pl. Vlll A and B).

> "Submerged aquatic fungus with branched, septate mycelium. Conidiophore sparingly branched often with two conidia at different stages of development. Conidium (aleuriospore) normally consisting of four divergent branches 20-40 mu. long, 2-3 mu. broad and of two, more or less spherical knobs, 3-5 mu. broad, one situated just above the point from which the four branches diverge, the other a short distance from this point on the upper side of one of these branches". (Ingold 1942).

Abnormal spores of the type described by Ingold (1942) from cultures of the fungus on malt agar have also been observed in water in which the fungus was growing on decaying leaves: one of these is illustrated in (Pl. Vlll,B).

In addition to Ingold's record of Tetracladium marchialanum from England (Ingold 1942) it has also been recorded recently from Switzerland (Ingold 1949), from Sweden (Nilsson 1958 and Willen 1958), from California

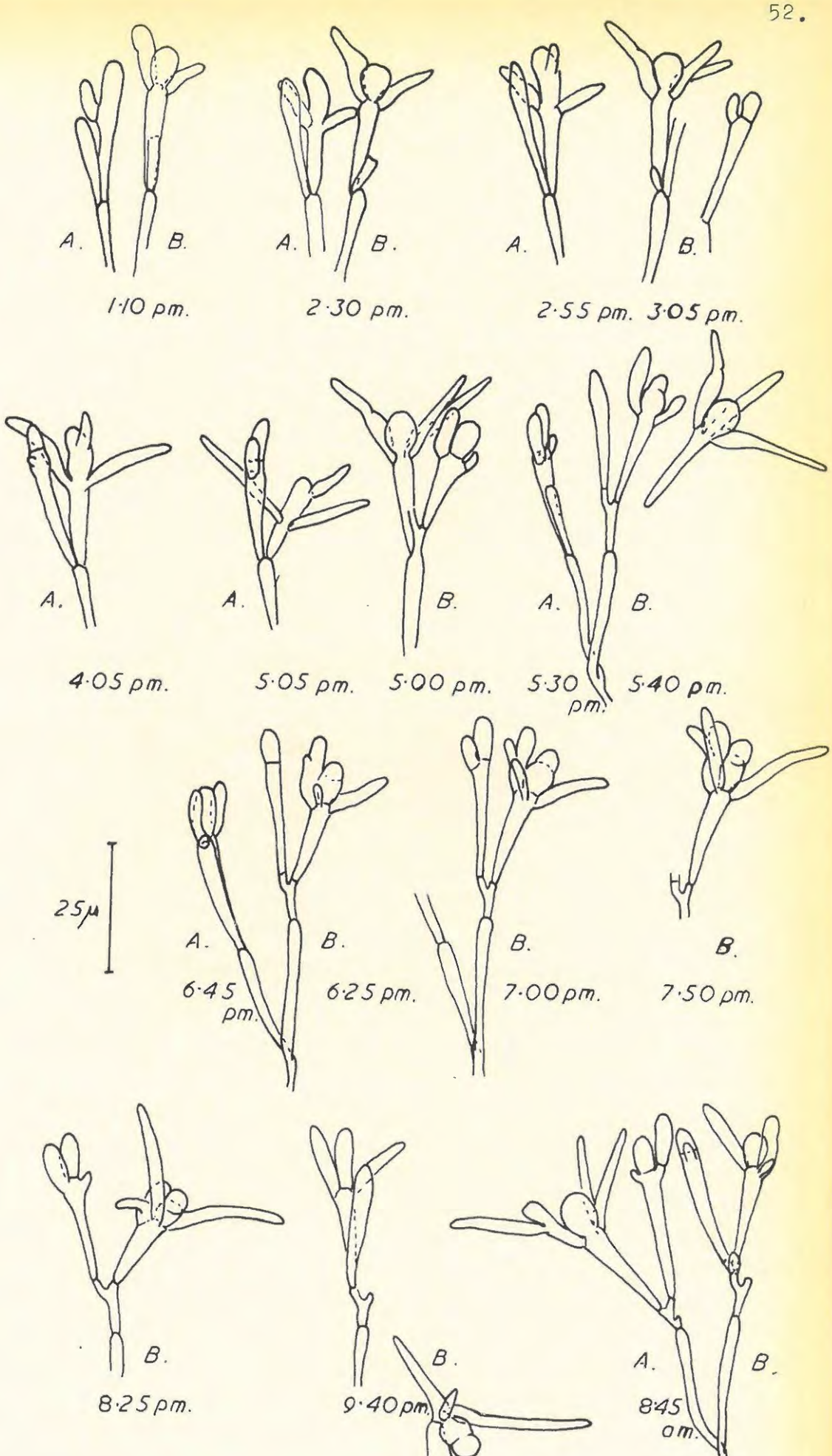


Fig. 12.

Tetracladium marchalianum De Wild. Spore development. From a hanging-drop culture of the fungus growing on a decaying leaf of *Populus nigra*.

(Ranzoni 1953), from Japan (Tubaki 1957), from Jamaica (Hudson and Ingold 1960) and from Rhodesia and Uganda (Ingold 1958). This fungus has been found growing on submerged leaf skeletons of the exotic trees, Populus nigra and Quercus robur from the Belmont Valley: Populus serotina and Quercus robur from the Hogsback: Salix babylonica from Port Elizabeth, of Combretum caffrum from the Tyumie River and on soft but not slimy leaf skeletons of the herb, Plectranthus ecklonii from the Hogsback. Conidiophores of this fungus were observed to arise more frequently from the mid-rib, lateral veins and petiole of leaf skeletons than from the leaf margin. They were found in greatest abundance in March, July and August, the autumn and winter months.

Spore development has been followed in hanging drop cultures of the fungus growing on decaying leaves of Populus nigra (Fig. 12). The spore primordium elongates and a small apical knob-like cell is cut off by a septum. A protruberance, which will form the second divergent arm on the second knob of the spore, appears just below this septum (Fig. 12,d). A second protruberance, which will form the third divergent process, appears on the opposite side of the knob (Fig. 12,g). On the side of the first protruberance there appears a third protruberance which will form the fourth divergent arm (Fig. 12,h,B). The mature spore is formed by further growth of its branches (Fig. 12,h-k,B) and is liberated by the apparent dissolution of the middle lamella of the wall separating it from the conidiophore (Fig. 12,l). The development of a spore from the formation of the primordium to the liberation of the spore takes about nine to ten hours.

Ingold (1942) noted that "while the developing spore is still young, usually when its first arm is beginning to grow out, the conidiophore gives rise to a branch a

short distance below the first spore. This branch is soon cut off by a wall near its base and is the primordium of the new spore". Then after the first spore is liberated "while the second spore is still developing a new hypha grows out from the stump from which the first has separated. This usually arises slightly to one side of the stump. It is soon cut off by a cross wall and is the primordium of the third spore". However, it was found that, though a branch may be produced a short distance below a developing spore, it may alternatively be produced slightly to one side of a developing spore, while it is still young. In the material examined it was more usual for a spore primordium to be produced next to a young spore than for it not to be developed in this position until after the first spore was shed (Fig. 12,a,g, A).

The spores germinate readily on two per cent malt agar made with stream water and pure cultures of the fungus have been isolated on this medium. The cultural characteristics are given in Part 3.

When strips of culture were transferred to sterile stream water, which was constantly aerated, spore primordia were produced abundantly within eighteen hours and after twenty two hours mature spores had developed. This agrees with Ingold's observation (1942) that under these conditions conidiophores were produced abundantly within twenty four hours.

Tetracladium setigerum (Grove) Ingold.
(Pl. VIII C, D and E).

"Aquatic fungus with branched septate mycelium. Conidium (aleuriospore) consisting of four divergent arms 20-40 mu. long, each tapering from 3 mu. near the central region of the spore to 1 mu. near its tip, and of three

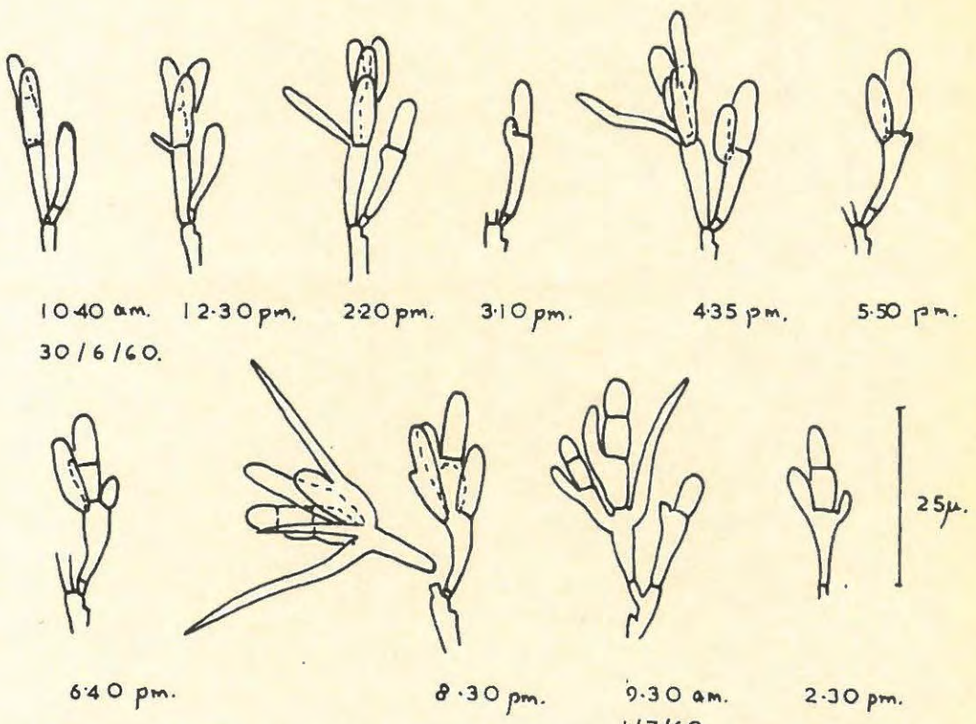


Fig.13. Tetracladium setigerum [Grove] Ingold. Spore development. From a hanging-drop culture of the fungus growing on a decaying leaf of Populus serotina.

elongated, parallel, finger-like processes, 12-15 mu. long, 3-9 mu. broad two of which are inserted just above the point of divergence of the four arms and the third a short distance along one of the arms". (Ingold 1942).

In addition to this record of Tetracladium setigerum from England it has been recorded recently from California (Ranzoni 1953), from Switzerland (Ingold 1949), from Sweden (Nilsson 1958 and Willen 1958), from Japan (Tubaki 1957), from Jamaica (Hudson and Ingold 1960) and from Uganda (Ingold 1958). This fungus has been found growing on submerged leaf skeletons of the exotic tree, Populus serotina from the Hogsback and of the indigenous trees, Maytenus cymosus from the Hogsback and Rhus legati from Fern Kloof. Conidiophores of this fungus were found in collections intermittently throughout the year, but never in abundance.

Spore development has been followed in a hanging drop culture of the fungus growing on a decaying leaf of Maytenus cymosus (Fig. 13) and the pattern of development confirmed that deduced by Ingold (1942) from an examination of conidia at various stages in their development. The first spore primordium is terminal on the conidiophore but, while this spore is still young, a short branch bearing a second primordium may be produced either beside the first spore or on a short branch which arises slightly below it. The primordium is cut off from the conidiophore by a septum and is in the form of a long, slightly swollen cell (Fig. 13,a). Towards the apex of this cell a septum forms cutting off a small apical cell (Fig. 13,c) which will give rise to two of the finger-like processes, and a large lower cell, which is the first divergent arm. Soon afterwards a small protruberance, which will give rise to the third finger-like process and one of the

divergent arms, appears slightly below and to one side of the septum (Fig. 13,d). As it grows, this process bends upwards, more or less parallel with the small apical cell, which is also elongating. The apical cell becomes constricted about half way along its length and a septum is formed at this level. Shortly afterwards slightly below this septum there appears a protruberance, which becomes the second finger-like process (Fig. 13,g). While the first finger-like process is elongating, a protruberance, which will form the third divergent arm, appears slightly below the septum cutting off the first process and on the side away from the third process (Fig. 13,f). A protruberance which will form the fourth divergent arm appears at the same level as the third but between it and the third finger-like process (Fig. 13,h). Shortly after this, on the third finger-like process about half way along its length on the side away from the other two, there appears a protruberance which will form the second divergent arm (Fig. 13,h). A septum forms just above this protruberance across the third finger-like process, which like the other finger-like processes may become further septate above the points of branching (Fig. 13,i). Liberation of the spore seems to take place, as in Tetracladium marchialanum, by solution of the middle lamella of the septum separating the spore from the conidiophore.

Articulospora Ingold.

"Aquatic fungi with branched, septate mycelium. Conidiophores and conidia (aleuriospores) produced below water. Conidium of four long divergent arms which develop in succession. There is a constriction where each of the three later-formed arms joins the first formed arm of the spore". (Ingold 1942).

Articulospora tetracladia Ingold.

(Pl. 1X, A,B).

"Submerged aquatic fungus with branched, septate hyaline mycelium. Conidium (aleuriospore) hyaline, of four divergent arms: the first formed arm 20-35 mu. long, 3 mu. broad, 1-2 septate, the other three arms 36-75 mu. long, 3 mu. broad, 1-3 septate, each with a narrow constriction or isthmus, where it joins the short arm. In the formation of the spore the four arms arise in succession. Conidiophore simple or branched, producing spores from its apex (or apices) in succession, not basipetal but side by side.

On decaying leaves of Alnus glutinosa from a stream in Leicestershire, England". (Ingold 1942).

Other records of Articulospora tetracladia are from California (Ranzoni 1953), from Switzerland (Ingold 1949), from Sweden (Nilsson 1958), from Japan (Tubaki 1957), from Jamaica (Hudson and Ingold 1960) and Uganda (Ingold 1958). This fungus has been found abundantly throughout the year on submerged leaf skeletons of the indigenous trees, Rhus legati from fern Kloof and Howiesons Poort: Podocarpus latifolius, Celtis africana and

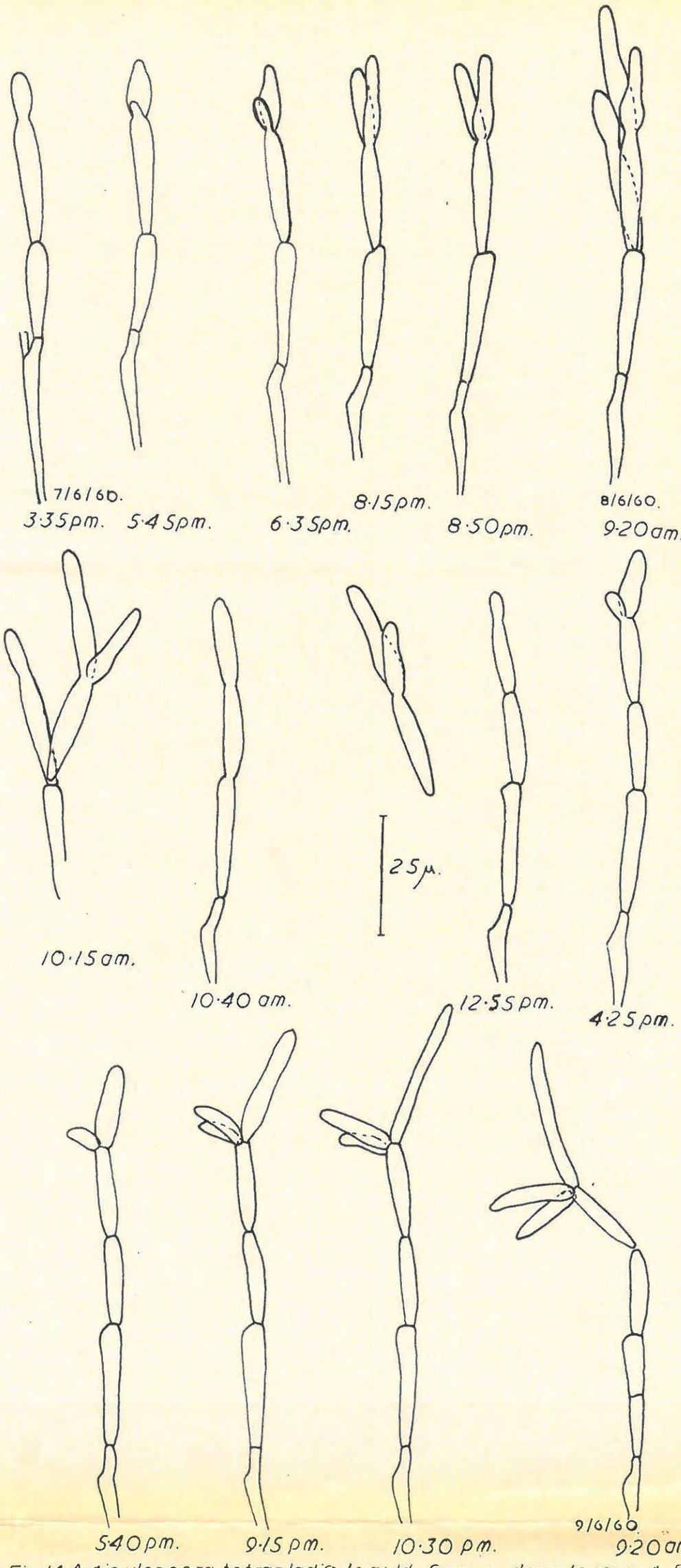


Fig. 14. Articulospora tetracladia Ingold. Spore development. From a hanging-drop culture of the fungus growing on a decaying leaf of Celtis africana.

Rapanea melanophloeos from Paradise Kloof: Celtis africana, Rhus legati, Maytenus cymosus and Royena lucida from the Hogsback: of the exotic trees Populus serotina and Quercus robur from the Hogsback: of Rubus sp. from the Hogsback and of the scrambling herb Urtica sp. from Fern Kloof. The conidiophores were found to arise more abundantly from the mid-rib, lateral veins and petioles than from the margins of the decaying leaves.

Spore development was followed in hanging drop cultures of the fungus growing on decaying leaves of Celtis africana (Fig. 14). The spore primordium develops as an outgrowth from the tip of the conidiophore and is cut off from the conidiophore by a septum. It then elongates and becomes constricted a short distance below its apex (Fig. 14,i). The portion below the constriction is the first arm and the portion above the constriction will form the second arm. Soon afterwards on the side of the first arm just below the constriction there appears a protruberance, which is constricted at its base and will form the third arm (Fig. 14,j). The fourth arm develops from a second protruberance, which appears to one side of the third arm just below the constriction between the first and second arms (Fig. 14,l). When the spore is mature, it is liberated by separation along the middle lamella of the septum between the spore and the conidiophore (Fig. 14, g,n,). The development of a spore under laboratory conditions, from the formation of the primordium to the liberation of the spore may take up to twenty two hours. This confirms the account of spore development by Ingold (1942). Spores with only three arms are sometimes developed (Fig. 14,g). Before the spore has completed its development, at one side of it on the end of the conidiophore, an new spore primordium is developed or an outgrowth produced which elongates and is divided by a septum into a spore primordium and a

conidiophore cell.

The spores germinate readily in water and on two per cent malt agar. A spore germinating in water was found in which two of the germ tubes had developed into conidiophores bearing conidia (Pl. 1X,B). These conidia were much smaller than those produced in the normal way.

Several isolations of this fungus have been made and the cultural characteristics are given in Part 3. When strips of the culture growing on malt agar, were immersed in water conidiophores were produced in abundance within twenty four to thirty three hours under laboratory conditions. The conidia produced agree with those formed by the fungus growing on leaves. These observations confirm those of Ingold (1942).

Articulospora grandis n.sp.

(Pl. 1X, C and D).

Submerged aquatic fungus with branched, septate, hyaline mycelium. Conidiophore hyaline, simple. Conidia (aleuriospores) produced in basipetal succession, hyaline, of four divergent arms: the first formed arm is 66-87 mu. long: 6-9 mu. broad at the base, 0.4 mu. broader at the junction with the other arms: 3-7 septate: the other three arms 55-100 mu. long, 9-11 mu. broad at the base tapering to 5-7 mu. broad at the distal ends: 3-7 septate: each constricted, where it joins the first formed arm. In the formation of the spore the four arms arise in succession.

Hab. On submerged decaying leaves from streams in the Eastern Cape Province of South Africa.

Articulospora grandis sp. nov.

Fungus aquaticus, submersus, mycelio ramoso septato, hyalino. Conidiophora hvalina, simplicia. Conidia (aleuriosporae) continua, exeodem loco deinceps enorientia, hyalina, quadriradiata, quattuor radiis diversis. Radius primarius 66-87 mu. longus, parte inferiore ad 6-9 mu. latus, parte superiore ad 0.4 mu. latior, 3-7 septatus. Radii ceteri 55-100 mu. longi, parte inferiore ad 9-11 mu. lati, ad apicem ad 5-7 mu. attenuati, 3-7 septati, unusquisque constrictus ubi radio primario conjungitur. In sporo formando quattuor illi radii continui deinceps evolvuntur.

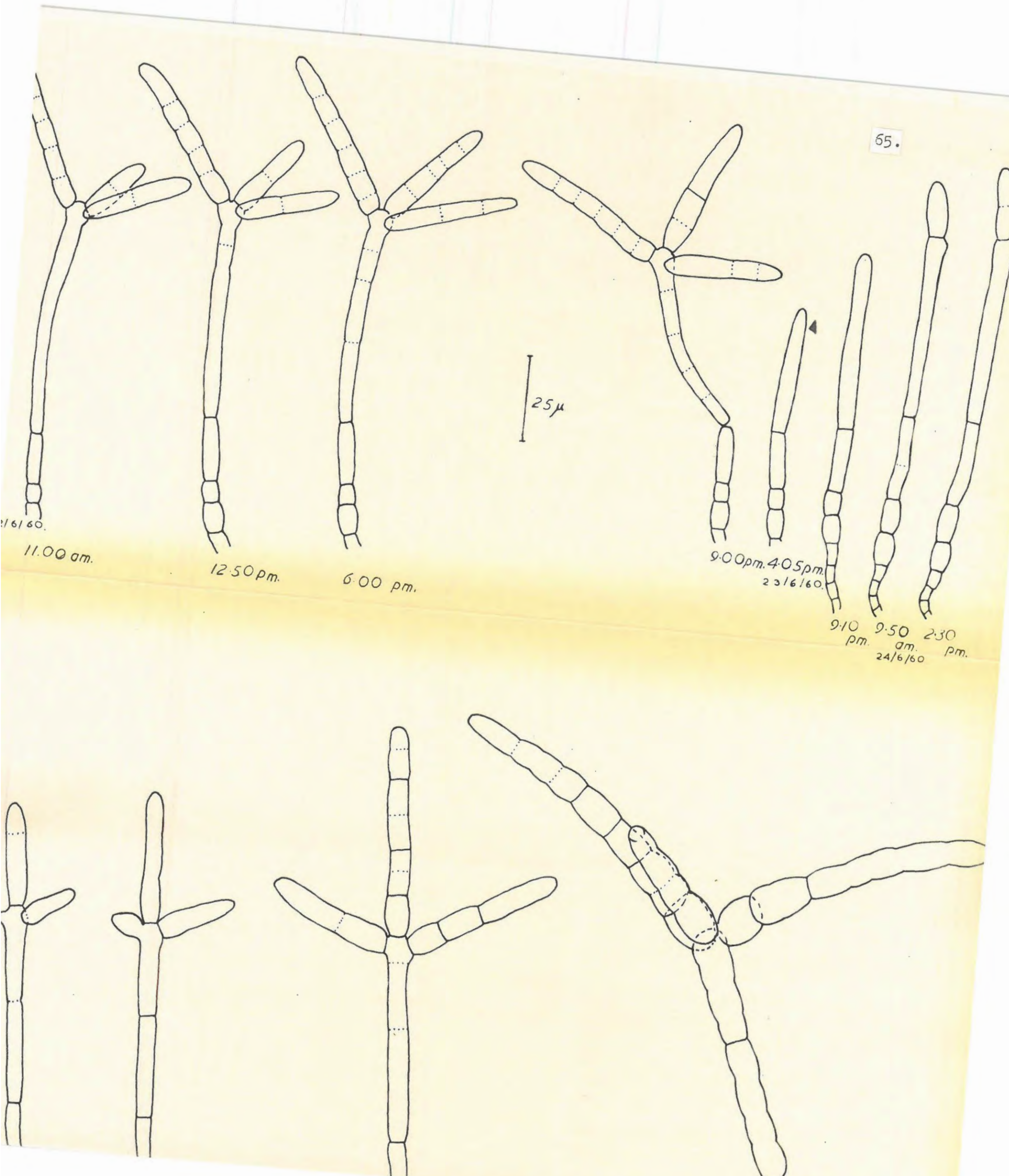
Hab. In foliis putrescentibus, in fluminibus in orientale parte Capicae Provinciae Africae Australis submersis.

Articulospora grandis was found throughout the year but was most abundant in the summer months, November and December, on submerged decaying leaves of Celtis africana and Podocarpus latifolius from Paradise Kloof and of Rapanea melanophloeos from Paradise Kloof and Howiesons Poort. The conidiophores in the material collected were produced on the mid rib and lateral veins of the decaying leaves.

This fungus agrees with the general description of the genus Articulospora (Ingold 1942) but differs from the described species. The spores are larger than those of Articulospora tetracladia and are produced in basipetal succession in this fungus and side by side from the end of the conidiophore in A. tetracladia. Although the lengths of the arms of the spores of A. grandis correspond to those of Articulospora inflata, the arms are much broader than those of A. inflata, 9-11 mu. broad at the

TABLE 4. TABLE OF COMPARISON OF ARTICULOSPORA SPP.

Articulospora spp.	main arm			secondary lateral arms.			Remarks.
	length in mu.	width in mu.	inflated at apex	length in mu.	width in mu.	constricted at base.	
A. tetracladia	20-35	3	not	36-75	3	"	spores produced side by side on conidiophore.
A. angulata	20-25	2.5-4.0	not	10-12	2.5-4.0	"	additional tertiary arms.
A. moniliforma	13-16	2.5-4.0	not	10-16	3-4	"	lateral arms obclavate
A. grandis	66-87	6-9	not	55-100	9-11	"	spores produced in basipetal succession.
A. inflata	50-70	3-4		50-120	3-4	2nd formed arm not c. at base.	spores solitary ,
A. sp.(unidentified)	97	7.5		119-167	6	"	spores solitary.


25μ
2/6/60.
11.00 am.
12.50 pm.
6.00 pm.
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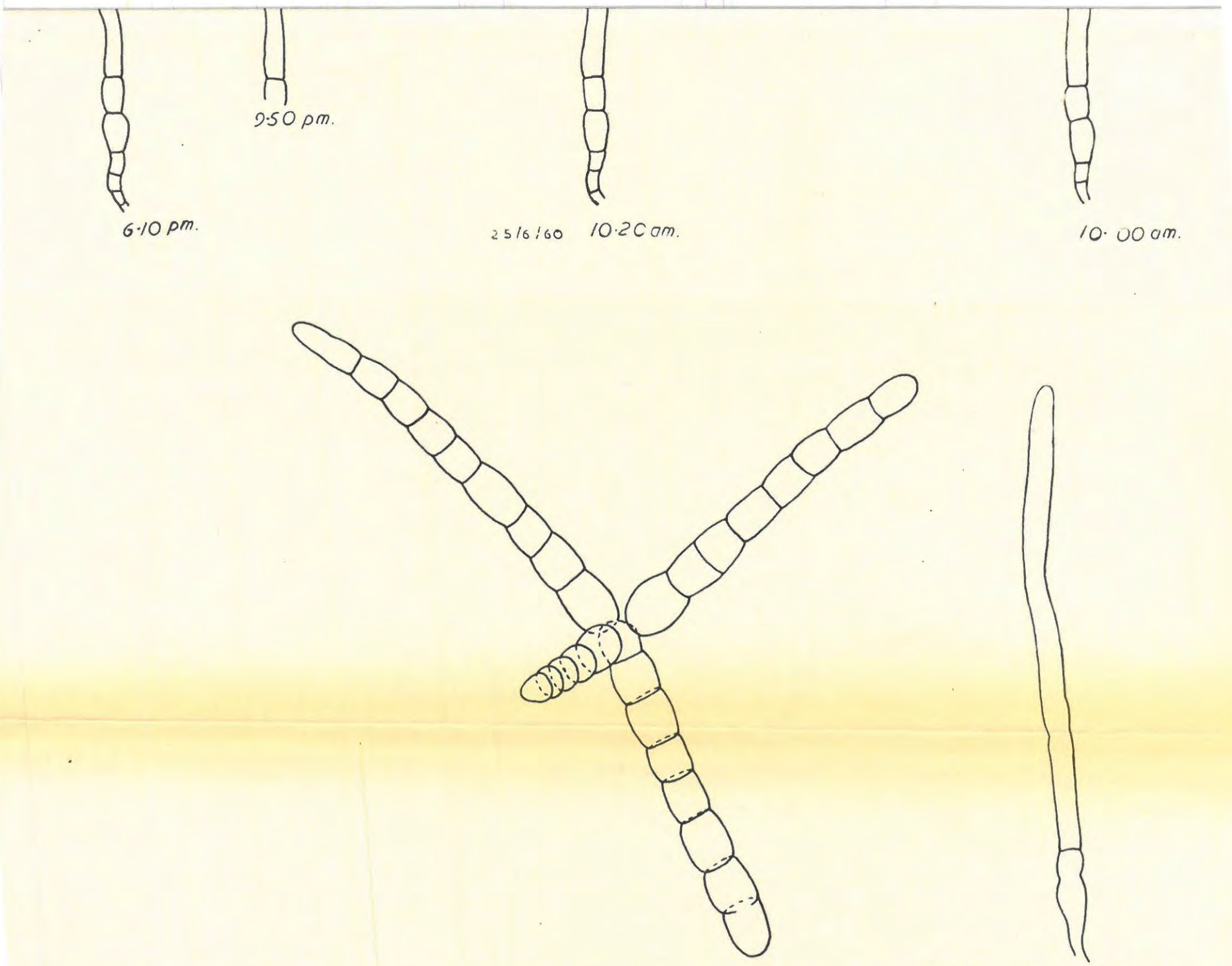


Fig.15.

Articulospora grandis n.sp. Spore development. From a hanging-drop culture of the fungus growing on a decaying leaf of *Celtis africana*

base and 5-7 mu. broad at the distal ends in the former as compared with 3-4 mu. in the latter (see Table 4). Further the second arm is constricted at the base in this fungus but not in A. inflata. The spores of A. grandis differ from those of Articulospora moniliforma Ranzoni in that they are much larger and the arms of the spore do not taper to a point at their distal ends as they do in the latter. Professor C.T. Ingold has, in a private communication, expressed the opinion that the spores of a fungus found by him in Rhodesia belong to this species. They are, however, somewhat smaller than those of this species described above.

Spore development has been followed in hanging drop cultures of the fungis growing on a decaying leaf of Celtis africana (Fig. 15). The spore primordium is cut off from the conidiophore by a septum (Fig. 15,e). It is a long cell approximately the same width as the conidiophore. The primordium elongates and becomes constricted a short distance below the apex. A septum is formed in this region cutting off the primordium of the second arm (Fig. 15,g). A protruberance, which will form the third arm, appears just below and to one side of the septum. As the protruberance grows out, it becomes constricted at its base and a septum forms across this constriction (Fig. 15,h). A second protruberance, which will form the fourth arm, appears below and to one side of the second arm but on the opposite side to the first protruberance (Fig. 15). This becomes constricted at the base and cut off by a septum which forms across the isthmus (Fig. 15,k). The four arms of the spore are now delimited about forty hours after the primordium was cut off from the conidiophore. The arms continue to increase in length and width, with the constriction at the bases of the second, third and fourth arms becoming more marked. The arms become constricted at intervals along their

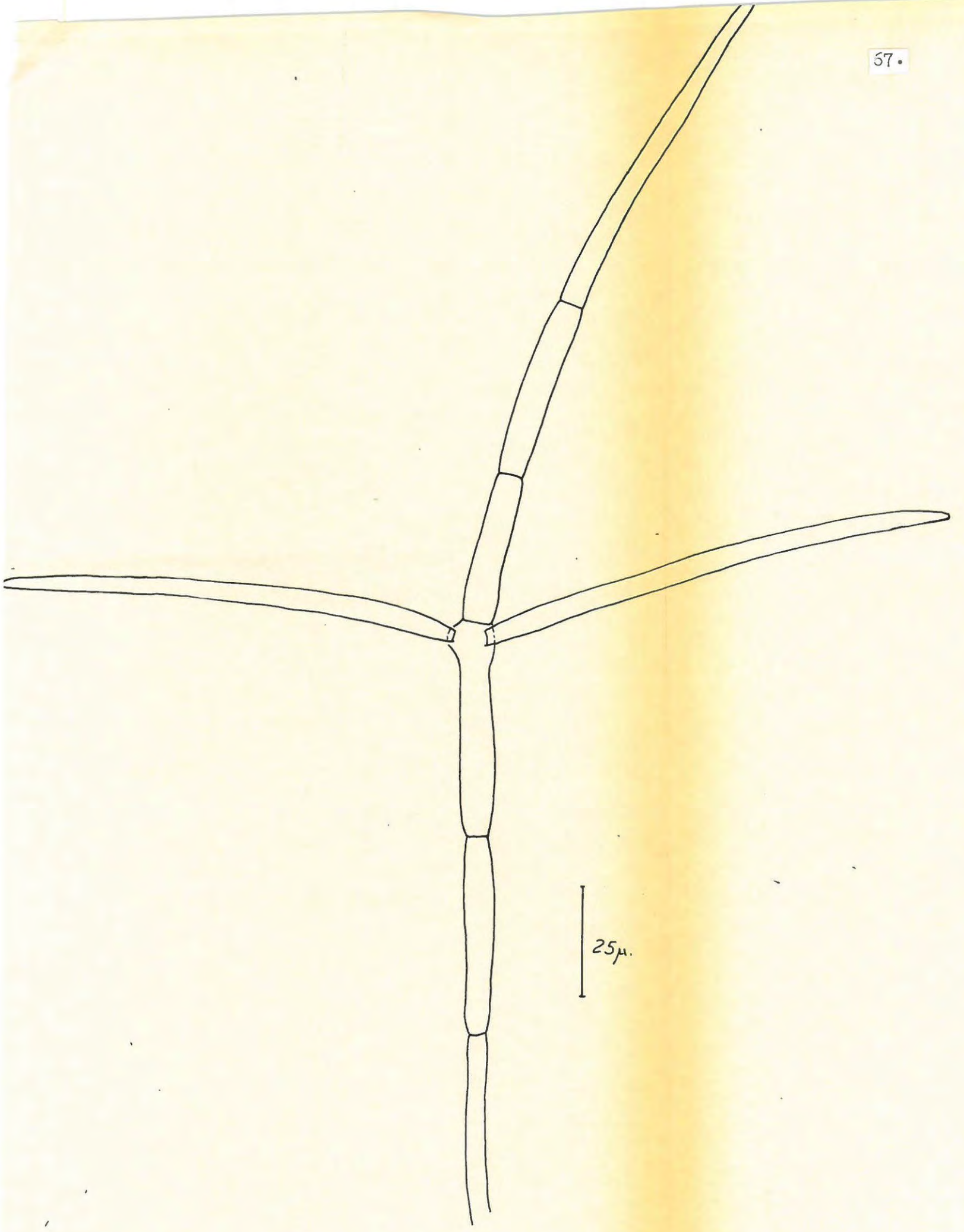


Fig. 16.
Articulospora sp. Growing on a decaying leaf of *Populus serotina* from the Hogsback. Mounted in water.

length and septa start to form across these constrictions (Fig. 15,k.l). The spore is then liberated by the rounding off of the tip of the conidiophore and of the basal end of the first arm sixty six hours after the formation of the primordium. After the spore has been liberated septum formation and increase in size continue (Fig. 15,m). The conidiophore elongates and another spore primordium is cut off but, unlike Tricladium splendens, no cells are added to the conidiophore. The spores germinate on two per cent malt agar.

Articulospora sp. (Fig. 16).

A few conidiophores and conidia were found which agreed with the general description of the genus Articulospora (Ingold 1942) but which did not seem to fit any of the described species, or the new species Articulospora grandis. The fungus was growing on leaf skeletons of Populus serotina from the Hogsback.

In one specimen the conidiophore was very long, 500 mu. long, 5 mu. broad and unbranched. The arm attached to the conidiophore was 97 mu. long, 7.5 mu. broad and slightly inflated in the region of its junction with the other arms, 11 mu. broad. The three lateral arms were 119-167 mu. long and 6 mu. broad at the base tapering slightly towards the distal ends. The arm in line with the main arm was hardly, if at all, constricted at the base, while the other two arms were slightly constricted at the base. For a comparison of the measurements with those of the other species see the table of comparison of the species (Table 4) of Articulospora.

PLATE X.

A.

B.

Alatospora acuminata Ingold.

A. Branched conidiophore with two phialides each bearing a conidium. The conidium on the right is almost mature. Growing on a decaying leaf of Maytenus cymosus from the Hogsback. Mounted in water.

B. Conidia in various stages of development. Growing on a decaying leaf of Combretum caffrum from the Tyumie River. Mounted in water.

Alatospora Ingold.

"Submerged aquatic fungi with branched, septate mycelium. Conidium (phialospore) consisting of four arms diverging from a common point. The development indicates that the spore consists of a curved main axis (forming two of the arms) and two laterals inserted at about the middle point of this axis". (Ingold 1942).

Alatospora acuminata Ingold.

(Pl. X).

"Submerged aquatic fungus with branched, septate mycelium. Conidiophore usually unbranched consisting of a single phialide 10–20 mu. long, 2–3 mu. broad, but often branched producing a group of two to four phialides. Conidium (phialospore) unicellular, hyaline, consisting of four divergent and approximately equal arms, 15–35 mu. long, 1.5–2.5 mu. broad, tapering towards their apices. Conidia produced in basipetal succession from the phialide.

On decaying leaves of Alnus glutinosa and Salix sp. from a stream in Leicestershire, England". (Ingold 1942).

Other records of Alatospora acuminata are from Switzerland (Ingold 1949), from Sweden (Nilsson 1958), from California (Ranzoni 1953), from Japan (Tubaki 1957), from Jamaica (Hudson and Ingold 1960) and from Rhodesia and Uganda (Ingold 1958). This fungus has been found growing on submerged decaying leaves of the exotic trees, Populus serotina, Quercus robur, Eucalyptus globulus and Acacia sp. from the Hogsback and of the indigenous trees and shrubs, Buddleja salvifolia, Celtis africana, Grewia occidentalis, Maytenus cymosus, Calodendrum capensis and

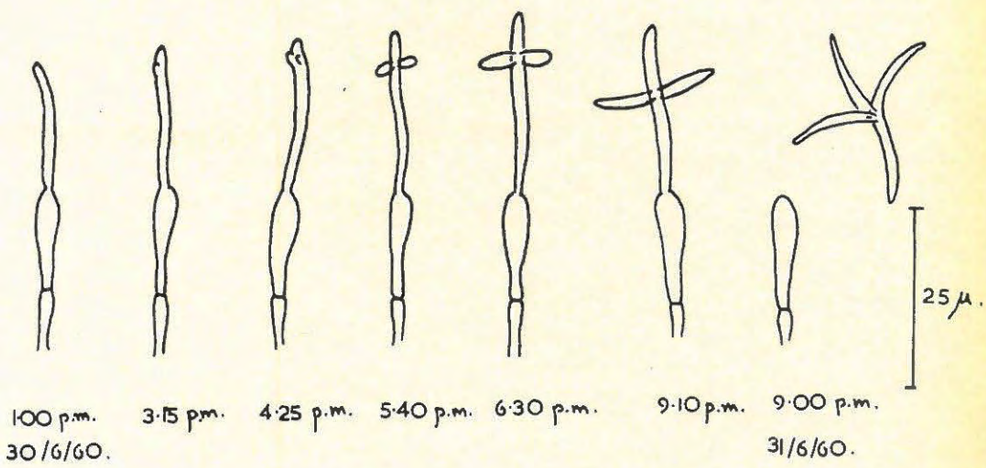


Fig 17. Alatospora acuminata Ingold. Spore development. From hanging-drop culture of the fungus growing on a decaying leaf of Maytenus cymosus.

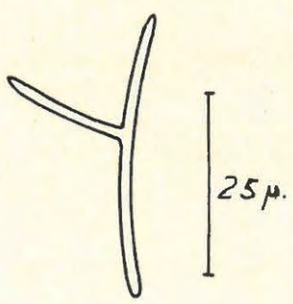


Fig. 18. Y-Shaped spore. Free-floating spore from the Blaauwkrantz River, Tzitzikama.

Podocarpus latifolius from the Hogsback: Rhus legati, Rapanea melanophloeos, Burchellia bubalina and Ficus capensis from Howiesons Poort: Celtis africana from Paradise Kloof and Combretum caffrum from the Tyumie River and of Rubus sp. and Polystichum sp. and on a decaying fruit of Acacia sp. from the Hogsback. Conidiophores of this fungus were found to arise most commonly from the margins of the decaying leaves. Alatospora acuminata was found abundantly during the autumn and winter months, March to August, decreased in abundance in the spring months, September and October, and was absent during the summer months, November to March.

Spores with five arms have been found to be produced, though not frequently, by this fungus growing on Maytenus cymosus.

Spore development has been followed in hanging drop cultures of the fungus growing on a decaying leaf of Maytenus cymosus (Fig. 17). The spore primordium grows out from the apex of the phialide as a straight hypha which bends over near its tip. Two bulges appear simultaneously close together on the convex curve of the hypha. These two bulges elongate to form two arms of the spore. This confirms the observations of Ingold (1942). The spores germinate on two per cent malt agar but attempts to obtain the fungus in culture have so far been unsuccessful.

"Y-shaped" spore. (Fig. 18).

Unidentified Y-shaped spores were found in water from the Blaauwkrantz River, Tzitzikamma Mountains, in December and from the streamlet in Fernkloof in March. The tail of the Y is 22 mu. x 2-2.5 mu. and the arms are 18 mu. x 2-2.5 mu.

PLATE XI.

Triscelophorus monosporus Ingold. Three conidiophores bearing conidia in various stages of development. The conidium in the centre is almost mature. Growing on a decaying leaf of Populus nigra from the Belmont Valley. Mounted in water.

Triscelophorus Ingold.

"Submerged aquatic fungi with branched, septate mycelium. Conidia (aleuriospores) terminal, branched, consisting of: (1) an elongated main axis continuous with the conidiophore, and (2) elongated secondary ramuli forming a whorl of three branches arising from the lower part of the main axis". (Ingold 1943).

Triscelophorus monosporus Ingold.
(Pl. XI).

"Submerged aquatic fungus with branched, septate, mycelium. Conidiophore hyaline, simple, straight, 15–45 mu. long, 1.5 mu. broad producing a single terminal conidium. Conidia (aleuriospores) hyaline, branched, aseptate (or with a single septum in the main axis just above the point of origin of the lateral branches): main axis continuous with the conidiophore, 50–70 mu. long, 4–5 mu. broad (at a distance of 3–5 mu. from the base) tapering to 1.5 mu. at the truncate base: secondary ramuli in a whorl of three, arising at a distance of 3–5 mu. from the base of the main axis, 40–50 mu. long, 2.0–2.5 mu. broad near the base tapering to 1.5 mu. at the apex, base abruptly constricted to 1 mu.

On a submerged decaying leaf from the river Loddon near Reading, England". (Ingold 1943).

Other records of Triscelophorus monosporus are from Sweden (Nilsson 1958), from California (Ranzoni 1953), from Japan (Tubaki 1957), from Jamaica (Hudson and Ingold 1960), from Nigeria (Ingold 1956), from Ghana (Dixon 1959)

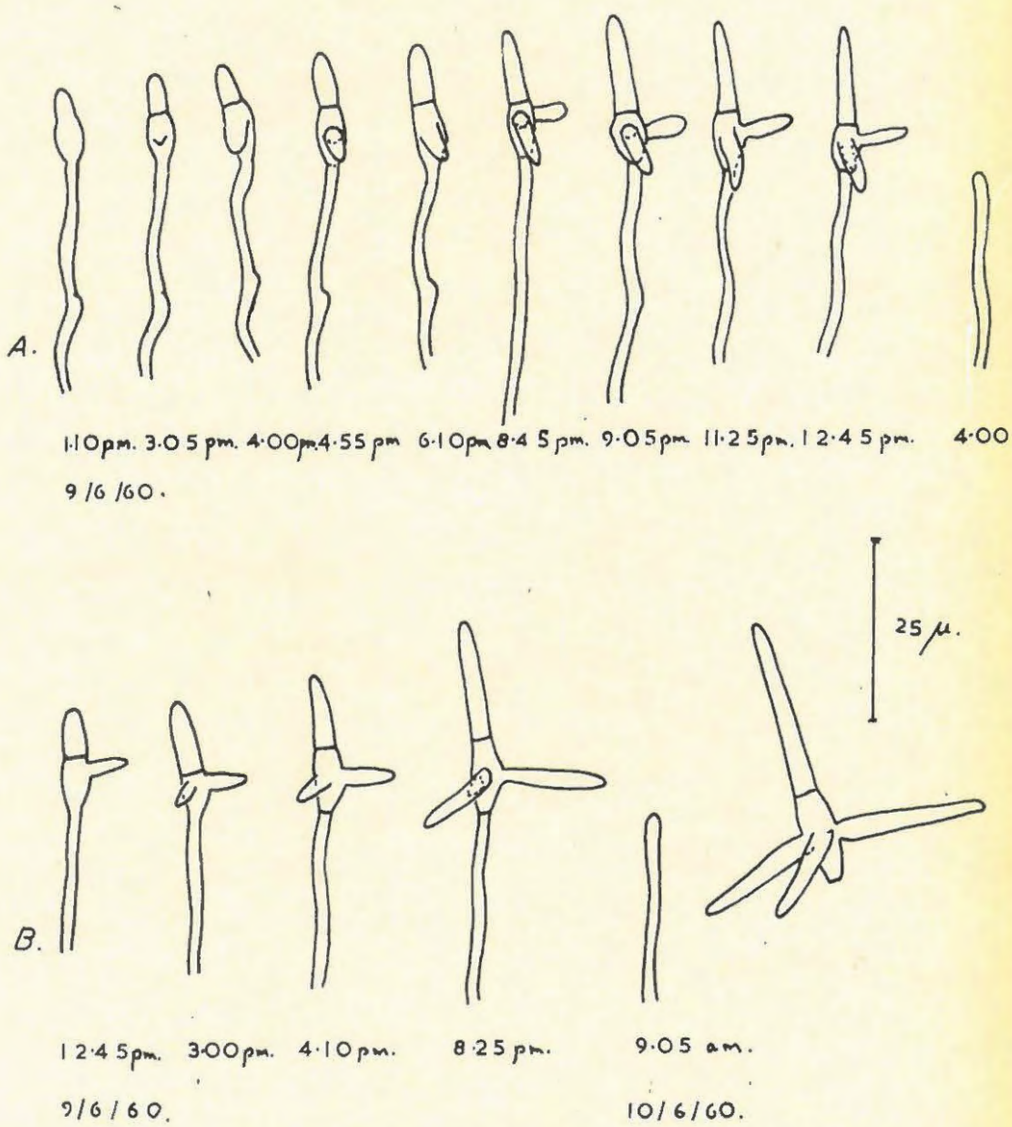


Fig.19 Triscelophorus monosporus Ingold. Spore development. From a hanging-drop culture of the fungus growing on a decaying leaf of Maytenus cymosus.

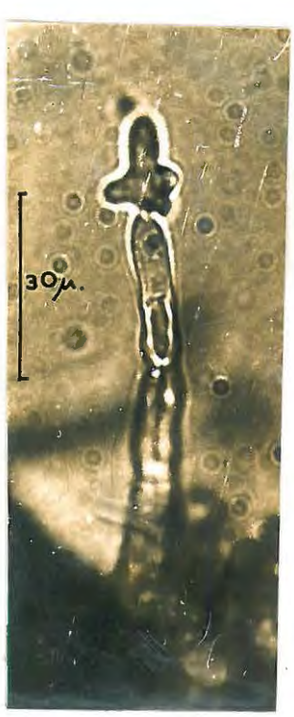
and from Rhodesia and Uganda (Ingold 1958). This fungus has been found growing on submerged decaying leaves of the exotic trees, Populus serotina, Eucalyptus globulus a and Acacia sp. from the Hogsback: Populus nigra from the Belmont Valley and Populus sp. and Quercus robur from Orinway: of the indigenous trees, Combretum caffrum from the Tyumie River: Podocarpus latifolius from the Hogsback, Rhus legati, Rapanea melanophloeos, Burchellia bubalina and Harpephyllum caffrum from Howiesons Poort and on decaying fruits of Acacia sp. from the Hogsback. The conidiophores are most frequently found on the margins of leaves which are not fully skeletonised. They are fairly abundant during the autumn and winter months, March to August, but are rare or absent during the spring and summer months, September to February.

Spore development has been followed in hanging drop cultures of the fungus growing on a decaying leaf of Rhus legati (Fig. 19). The spore primordium starts as a swelling at the tip of the conidiophore. The swelling enlarges and becomes differentiated into a wider lower portion and a slightly narrower upper portion (Fig. 19, a). These two parts become separated by a septum. From the middle of the lower portion a protruberance appears and grows outwards to form one of the three lateral branches (Fig. 19,b). The upper part of the spore continues to elongate. A second protruberance, which will form the second lateral arm, arises at the same level but at an angle of about 120° from the first (Fig. 19,f). At about this stage in its development the spore is cut off by a septum. The third lateral branch arises as a protruberance at angles of 120° from both of the other branches. The spore increases in size and is liberated, when fully grown, by the separation of the middle lamella of the cross wall

between the spore and the conidiophore (Fig. 19,o). This confirms the account given by Ingold (1943) except that he found that the spore primordium was cut off from the conidiophore early in its development, whereas it would appear that the septum is not necessarily formed until after the second of the three lateral branches has been formed.

PLATE XII.

A.



B.

C.

A. Lemonniera aquatica De Wild. Branched conidiophore. Phialides bearing conidia in various stages of development. Growing on a decaying leaf of Populus serotina from the Hogsback. Mounted in acid fuchsin and lactophenol.

B.&C. Lemonniera brachycladia Ingold. Two conidiophores with phialides bearing conidia in various stages of development. Growing on a decaying leaf of Celtis africana from the Hogsback. Mounted in water.

Lemonniera De Wild.

The following is a translation from the latin description by De Wildeman (1894, cited in Saccardo 1899):

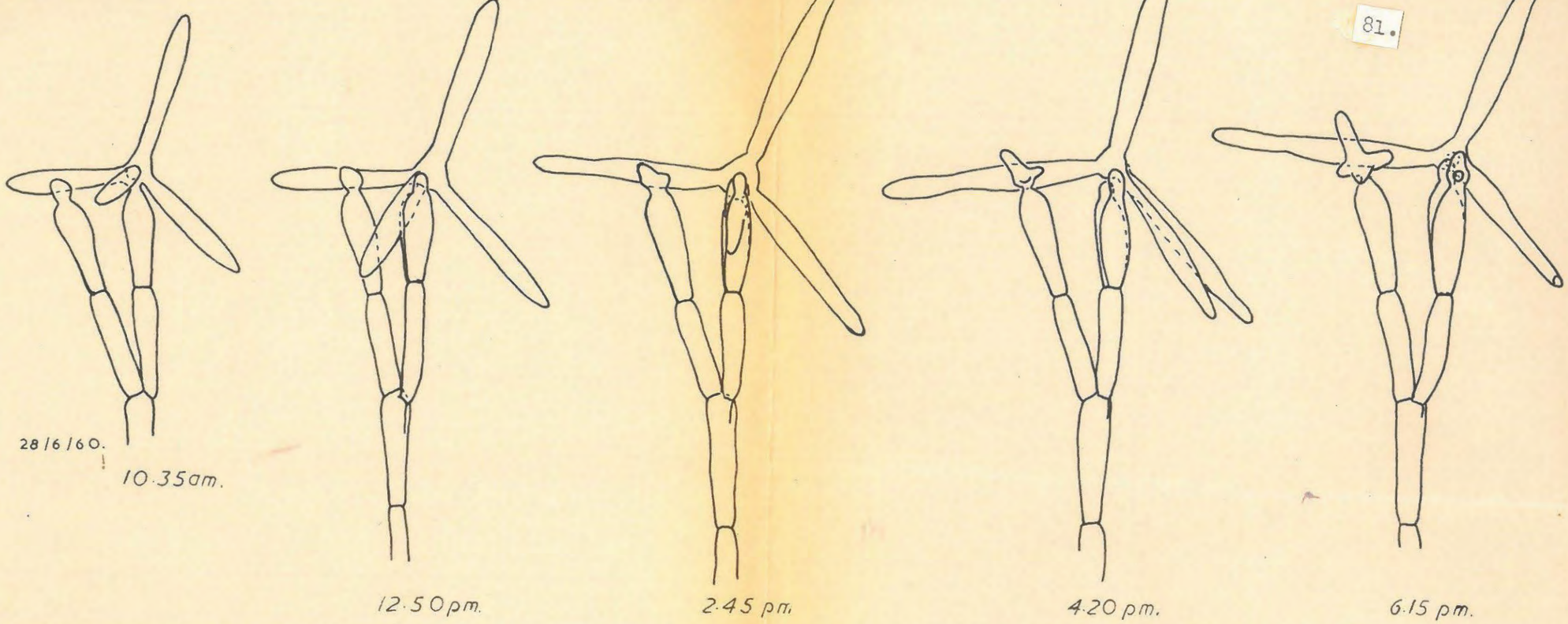
> Saprophytic, with multicellular mycelium emerging from and embedded in plant tissue: fertile hyphae erect, multicellular, branched, bearing quadriradiate conidia from the apex.

Lemonniera aquatica De Wild.

(Pl. XII,A).

> "Submerged aquatic fungus with branched, septate mycelium: conidiophore consisting of a straight unbranched portion which branches near its free end to form a group of two to eight phialides. Each phialide produces conidia (phialospores) in basipetal succession. Conidium consisting of four long divergent arms (which usually become septate), 20-70 mu. long, 3-4 mu. broad, and inserted on the phialide at the point of divergence of the four arms of the spore". (Ingold 1942).

The recent records of Lemonniera aquatica are from England (Ingold 1942), from Switzerland (Ingold 1949), from Sweden (Nilsson 1958 and Willen 1958), from Germany (Klotter 1955 cited in Willen 1958), from California (Ranzoni 1953) and from Japan (Tubaki 1957). This fungus was found growing on submerted skeletonised leaves of the exotic tree, Populus serotina, and of the indigenous trees, Xymalos monospora and Maytenus cymosus, from the Hogsback. The conidiophores are produced at right angles to the main veins of the decaying leaf. They were present in collections from April to August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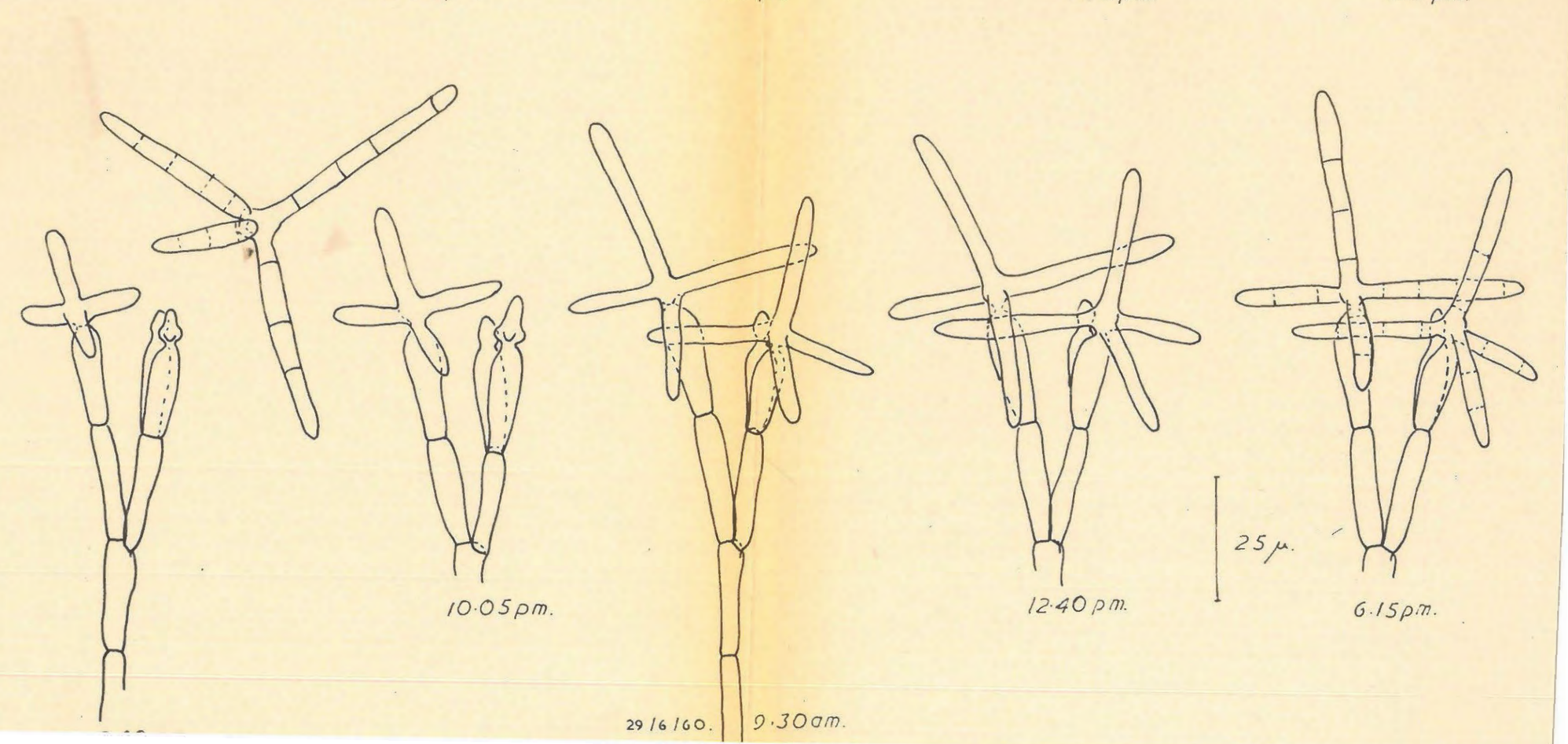
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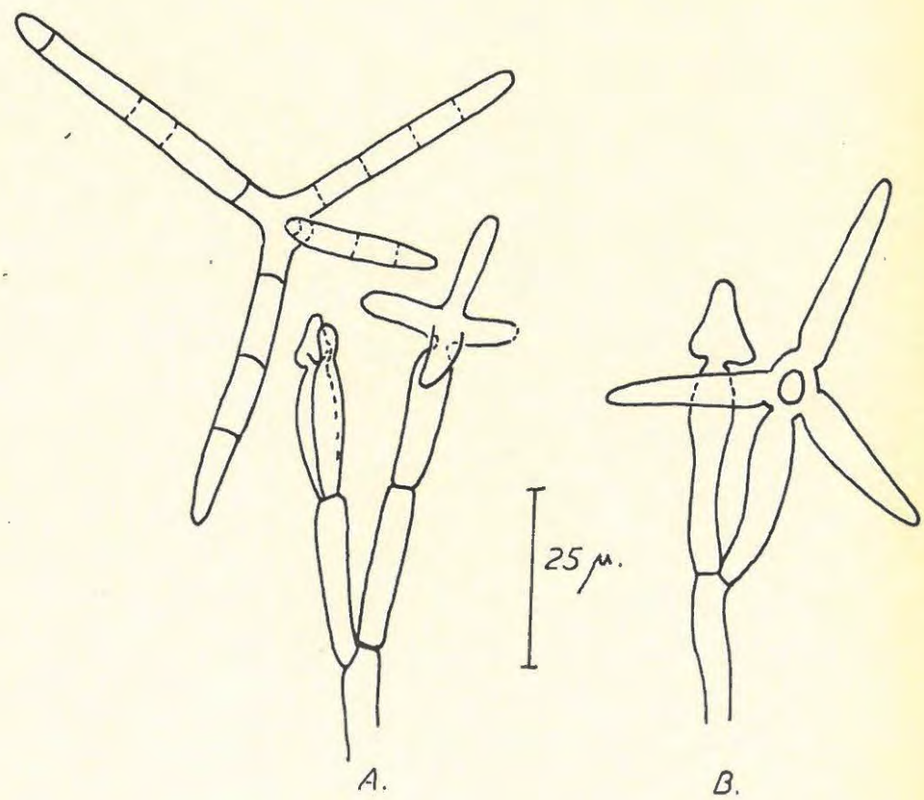

Fig.21. A. Lemonniera aquatica De Wild. Conidiophore growing from a decaying leaf of Populus nigra var italica from the Hogsback.

B. Lemonniera brachycladia Ingold. Conidiophore growing from a decaying leaf of Celtis africana from the Hogsback.
A and B mounted in water.

but only showed abundance during August.

Spore development has been followed in hanging drop cultures of the fungus growing on a decaying leaf of Populus serotina (Fig. 20). The spore primordium starts development as a small swelling at the end of the phialide (Fig. 20,d). This swelling enlarges (Fig. 20, a). becomes tetrahedral (Fig. 20,b,c) and the four arms grow out simultaneously from the corners of the tetrahedron (Fig. 20,d). When the spore is fully developed, a line of cleavage forms separating it from the phialide and it is soon liberated. Just before (Fig. 20,j) or just after (Fig. 20,f) liberation the arms of the spore become septate. Immediately after the spore has been liberated a new spore primordium is formed from the tip of the phialide (Fig. 29,d). This account of spore development confirms that of Ingold (1942).

Lemonniera brachycladia Ingold.
(Pl. XII B & C)(Fig. 21).

The following is a translation from the latin description by Ingold (1958):

> Submerged aquatic fungus, mycelium branched, septate, hyaline at first, later brown. Conidiophores hyaline, septate, ending in one or more phialides. Conidium (phialospore) hyaline, consisting of four equal diverging branches arising from the first formed central portion. Each branch 15-50 mu. long, 4-6 mu. broad near the base narrowing to 3 mu. at the apex, mostly septate, rarely uniseptate. Hab. On decaying leaves of Alnus glutinosa etc. in running streams, Ide Hill, Kent, England.

Lemonniera brachycladia was found growing on submerged leaf skeletons of the exotic tree, Populus serotina, and of the indigenous trees and shrubs, Rhus legati, Calodendrum capensis and Buddleja salvifolia from the Hogsback. This fungus, like Lemonniera aquatica, bears its conidiophores projecting from the main veins of the decaying leaf and produced its conidia during the winter months, March to August, and most abundantly during August.

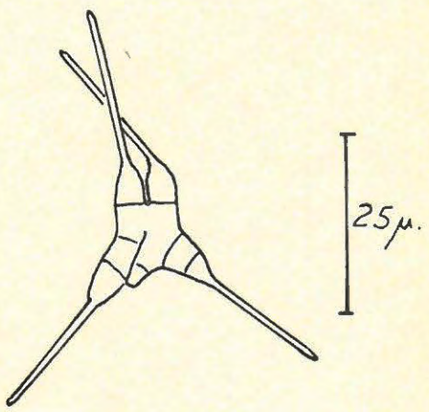

Fig.22. Campylospora chaetocladia Ranzoni

Free floating spore from a stream in the upper natural forest, Hogsback. Mounted in water.

Campylospora Ranzoni.

"Submerged aquatic fungi with branched, septate, mycelium. Aleuriospores septate, colourless, asymmetric, consisting of a basal cell with two divergent appendages and a lateral branch with two divergent appendages". (Ranzoni 1953).

Campylospora chaetocladia Ranzoni.
(Fig. 22).

"Submerged aquatic fungus with branched, septate, colourless mycelium. Aleuriophores colourless, unbranched, 10-20 mu. long, 2-2.5 mu. broad. Aleuriospores colourless, terminal, multicellular, each consisting of a basal cell 8.5-12 mu. wide, 10-14 mu. long, with two divergent appendages of approximately the same length, 35-50 mu. long, 6-3 mu. wide at the point of attachment to the basal cell and tapering to about 1.5 mu. at the tips: and a lateral branch 10-25 mu. long attached perpendicularly to the transverse axis of the aleuriospore and bearing at each end an appendage similar in appearance to those on the basal cell. Aleuriospores produced in succession but not basipetally. Hab. On decaying submerged leaves of Acer sp. in a stream in Sonoma County, California". (Ranzoni 1953).

In addition to this record of Campylospora chaetocladia from California it has been recorded from Jamaica (Hudson and Ingold 1960), from Nigeria (Ingold 1956), from Ghana (Dixon 1959) and from Rhodesia and Uganda (Ingold 1958). Spores of this fungus have been found associated with decaying leaves from a stream

in the upper natural forest at the Hogsback. The spores were never abundant but were found most frequently in April 1961.

4. A KEY TO THE SOUTH AFRICAN SPECIES OF AQUATIC HYPHOMYCETES.

This key is merely intended as a guide to the identification of those aquatic Hyphomycetes described in this thesis. It is probable that the key represents only a small number of the fungi of this type which occur in South Africa. Articulospora inflata is included in the key as spores have been found which might have belonged to this fungus.

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1. Spores branched.....(7)
   Spores unbranched.....(2)
2. Spores crescent-shaped.....Lunulospora curvula.
   Spores not crescent-shaped.....(3)
3. Spores unicellular or bicellular: phialospores....(4)
   Spores multiseptate: aleuriospores.....(5)
4. Spores less than 60 mu. long .....
   .....Flagellospora penicillioides.
   Spores greater than 100 mu. long.....
   .....Flagellospora curvula.
5. Spores 5-6 mu. broad in middle region tapering towards both ends: no projection at the base.....
   .....Anguillospora longissima.
   Projection at base of spore.....(6)
6. Spores 3.8 mu. broad tapering slightly at the apex only.....Anguillospora filiformis.
   Spores 6 mu. broad in the middle region tapering rapidly to 1.5 mu. at the apex and gradually to 4.5 mu. at the end near the projection.....
   .....Anguillospora sp.
7. Spores Y-shaped....."Y-shaped" spore type
   Spores not as above.....(8)

8. Spores consisting of a main axis with two branches arising at different levels.....(9)
Spores not as above.....(10)

9. Main axis 6-7 mu. broad at widest part.....
.....Tricladium splendens.
Main axis 3.0-3.5 mu. broad at widest part.....
.....Tricladium gracile.

10. Spore consisting of a clavate portion from which branches arise at the broad end.....(11)
Spore not as above.....(12)

11. Clavate part of spore 30-40 mu. long.....
.....Clavariopsis aquatica.
Clavate part of spore 10-20 mu. long.....
.....Heliscus longibrachiatus.

12. Spore asymmetric, consisting of a basal cell with two divergent appendages and a lateral branch with two divergent appendages.....
.....Campylospora chaetocladia.
Spores not as above.....(13)

13. Spore with branches of more than one type.....(14)
Spore with branches all of one type.....(15)

14. Spore consisting of four divergent arms and three finger-like processes.....
.....Tetracladium setigerum.
Spore of four divergent branches and two more or less spherical knobs..Tetracladium marchialanum.

15. Spore with one of the arms bearing the other three arms at its apex.....(16)
Spore not as above.....(19)

16. Arm bearing the other three arms, inflated near its apex.....(17)
Arm bearing the other three arms not inflated at its apex.....(18)

17. Arms of spore 3-4 mu. broad.....
.....Articulospora inflata.

Arms of spore 6-7.5 mu. broad.....
.....Articulospora sp.

18. Branches of spore 36-75 mu. long, 3 mu. broad spores produced side by side at the apex of the conidiophore.....Articulospora tetracladia.
Branches of spore 55-100 mu. long, 9-11 mu. broad at the base: spores produced singly from the apex of the conidiophore.....
.....Articulospora grandis.

19. Spore consisting of a curved axis bearing a pair of opposite arms.....Alatospora acuminata.
Spores not as above.....(20)

20. Spore consisting of a bicellular main axis somewhat swollen at the base bearing a whorl of three branches at the widest part.....
.....Triscelophorus monosporus.
Spores not as above.....(21)

21. Arms of spore with parallel sides.....
.....Lemonniera aquatica.
Arms of spore with sides converging towards the free ends.....Lemonniera brachycladia.

PART II.

ECOLOGICAL OBSERVATIONS.

An investigation has been made of decaying plant material from streams, stagnant pools in dry river beds, ponds and dams. The aquatic Hyphomycetes described in this thesis were found in streams but not in any of the other habitats. The only stream site in which they were not found was the Blaauwkrantz River, a mile or two below the outflow of the sewage effluent into the stream. A bacterial count was made at this site and it was found to be considerably higher than counts at sites in which these fungi flourish. As conidial production in these fungi has been observed to be most abundant on leaf skeletons which are free from bacteria, it seems probable that the large bacterial population at this point in this stream may be responsible for these aquatic Hyphomycetes being absent from an otherwise apparently suitable site. The fall in the production of conidia by the fungi on leaves kept in the laboratory, particularly, when they were in unaerated water was accompanied by an increase in the bacterial population. It would therefore appear that the reason for these fungi showing a preference for well aerated running water is in part due to the bacterial population being smaller than in poorly aerated stagnant water.

The pH of the water of most of the streams, where these fungi have been collected, has been measured and is on an average about 7.0. Table 5 shows the variation in pH during the year April 1960 - April 1961. It will be seen that the lowest pH, 6.0, was recorded from the stream in the oak plantation at the Hogsback in April but that during the other months during which the pH was measured it was greater than 7.0. The highest pH, 8.3, was recorded from the stream in one of the poplar plantations at the Hogsback in May but the pH at this site

was less than 8.0 during the other months during which it was measured. There thus seems to be little variation in the pH of the water of the streams investigated.

There does not seem to be a tendency for any one of these fungi to become established on a leaf skeleton to the exclusion of the others. In material collected from the Hogsback as many as eight species of these fungi have been found growing on a square centimetre of leaf skeleton of Populus serotina - Tricladium gracile, Clavariopsis aquatica, Heliscus longibrachiatus, Anguillospora longissima, A. filiformis, Lunulospora curvula, Alatospora acuminata and Triscelophorus monosporus - and as many as seven species - Tricladium gracile, Clavariopsis aquatica, Anguillospora longissima, Tricladium splendens, Flagellospora curvula, Alatospora acuminata and Lunulospora curvula - have been found growing on one square centimetre of skeletonised leaf of Rubus sp. from the Hogsback. It is, however, more usual to find from one to three species on a leaf skeleton.

Decaying leaves of a wide range of plants are colonised by these aquatic Hyphomycetes - a fern, Polystichum sp., a gymnosperm, Podocarpus latifolius, leaves of herbs such as Plectranthus ecklonii which form soft leaf skeletons, leaves of trees such as Celtis africana and Rhus legati with relatively thin cuticles which disintegrate during skeletonisation and of other trees such as Eucalyptus globulus, that have thick cuticles which persist when the leaf is skeletonised. Some of these fungi have also been found growing on decaying fruits of Acacia sp. in which the veins were exposed. The occurrence of these fungi on leaves of a gymnosperm is of particular interest, as in other accounts of these fungi attention has been drawn to the fact that all gymnosperm material examined was devoid of these fungi. The fungi inhabiting decaying leaves of Podocarpus latifolius are

Flagellospora curvula, Tricladium gracile, Articulospora tetracladia, A. grandis, Alatospora acuminata and Triscelophorus monosporus. Not only does this group of fungi as a whole inhabit a wide range of decaying plant material but the individual species also show a lack of specificity in their choice of a substrate (see Table 2). The best example is Alatospora acuminata which grows on the whole range of plant debris categories listed above.

The position in which the conidiophores are produced on a decaying leaf varies. The conidiophores may arise at right angles to the petioles, veins or leaf margins. Conidiophores occur with equal frequency on veins and leaf margins in Tetracladium setigerum, Flagellospora curvula and Tricladium gracile, on both veins and margins but more frequently on veins in Lunulospora curvula, most frequently on the margins of leaves which are not fully skeletonised in Triscelophorus monosporus, Alatospora acuminata and Heliscus longibrachiatus and more frequently on veins and petioles than on margins in Clavariopsis aquatica, Lemonniera aquatica, Lemonniera brachycladia, Anguillospora longissima, Tricladium splendens, Tetracladium marchialanum, Articulospora tetracladia and A. grandis.

As these fungi were only collected during the period of one year, April 1960 to April 1961, no definite statements can be made as to seasonal fluctuations. It may, however, be of interest to compare some of the observations with those of Ingold (1942) for the same species growing in England. Observations for several species show a correspondence with Ingold's observations. Lunulospora curvula was most abundant in autumn, April and May, but showed a marked decrease during the winter months. Ingold found it to be very abundant especially during the late summer and autumn but to be rather rare during the winter months. Alatospora acuminata was

found most abundantly during the winter months, July and August, but was less common at other times of the year. Ingold found that in England it was especially common during the winter and spring but much less frequent during the summer and autumn. Articulospora tetracladia was observed to be common throughout the year and Ingold made the same observation in England. Observations for some of the species do not however correspond with Ingold's observations for them in England. Lemonniera aquatica, Heliscus longibrachiatus, Tricladium splendens and Articulospora longissima were found during the winter months, April to August, August, July and August, and May to August respectively, whereas in England Ingold found them throughout the year. If these observations are in fact a true reflection of the seasonal fluctuation in South Africa, it would appear that sporulation, which in many species occurs throughout the year in England, is limited to the winter months in South Africa. This might possibly be a temperature effect due to the summer temperatures in South Africa being greater than those in England. It does not seem to be due to seasonal availability of substrate, as suitable leaves were present in the streams during the summer.

PART III.

aerial mycelium. The whole colony loses its greenish tinge with age and becomes a brownish black. Ingold (1942) described a dark olive green colony with a white fringe and Ranzoni (1953) described a brownish black colony with a white border. It seems as though this variation in the colour might be due to the age of the colonies described.

(ii) Czapek agar. The colony is compact, convoluted, dark greenish grey on the reverse side with a white margin. Little aerial mycelium is produced.

(iii) Maize agar. The mycelium is sparse and very little of it is aerial. The colour shades from dark grey in the older central portion of the colony to light grey at the edges.

(iv) Plain agar. The entire colony is very scant and colourless. No aerial mycelium is produced.

(c) Tetracladium marchialanum De Wild.

Several isolates of this fungus were made and all were similar in appearance.

(i) Malt agar. The colony is pinkish cream as compared with the white to yellow colony produced by Ingold's (1942) isolates of this species. Yellow staining is however apparent in irregular patches on the reverse side particularly at low temperatures. The surface of the colony is smooth except in the older parts, where a white cottony aerial mycelium is produced.

(ii) Czapek agar. The colony is white to cream, compact, radially convoluted, the surface is velvety and zonation is lacking. Yellow staining is apparent on the reverse side particularly at low temperatures.

(iii) Maize agar. The colony is zoned and the mycelium is colourless, sparse and a small amount of white aerial mycelium is produced.

(iv) Plain agar. The colony is indistinctly zoned and is colourless.

(d) Articulospora tetracladia Ingold.

Three isolations of this fungus have been made. All were similar in appearance but varied slightly in the depth of colour and the amount of aerial mycelium produced.

(i) Malt agar. The colony is compact, zoned with the reverse a deep yellow-ochre to buff in the central region shading to pale cream at the margin and there is a variable amount of aerial mycelium which is either a deep yellow-ochre to buff or is whitish. Ranzoni's (1953) isolates were buff coloured and those of Ingold (1942) were white. The colour would therefore appear to be variable.

(ii) Czapek agar. A very compact, much convoluted, deep yellow-ochre to orange colony is formed. It has a velvety surface and slight zoning is apparent near the margin.

(iii) Maize agar. A very sparse colourless to whitish, zoned colony with little aerial mycelium is formed.

(iv) Plain agar. A very sparse colourless to whitish, zoned colony with no aerial mycelium is formed.

(e) Clavariopsis aquatica De Wild.

(i) Malt agar. The colony is compact with an extensive aerial mycelium at all temperatures up to 30°C. at which temperature it is less abundant. The reverse is dark grey in the

older, central portion shading to whitish towards the margin which is indistinct. The dark grey region is limited in extent at 5° and 30°C. but predominant at the intermediate temperatures. The aerial mycelium is in all cases white to greyish. Ingold (1942). describes the colony on malt agar as being dark olive green in the older parts but with an irregular white fringe. In his isolates there was also much growth of white aerial hyphae over the whole surface of the colony.

(ii) Czapek agar. A compact, whitish colony with little aerial mycelium is formed.

(iii) Maize agar. The colony is generally colourless, not zoned and a little white to greyish aerial mycelium is produced in the older parts. At 5°C. the colony is, however, compact and white.

(iv) Plain agar. A very sparse colourless colony is produced.

(f) Flagellospora penicillioides Ingold.

(i) Malt agar. The colony is colourless at first becoming rust brown and later dark brown in the older central region. A little aerial mycelium is produced in the older parts and contains moniliform chains of brown cells. This isolate resembles those described for this species by Ingold (1942) and Ranzoni (1953).

2. GROWTH IN LIQUID CULTURE.

Four fungi Tricladium gracile, Tricladium splendens, Tetracladium marchialanum and Articulospora tetracladia were grown in liquid culture using Czapek-Dox medium. The method of preparation of the medium is outlined in Appendix 1. 250 c.c. flasks each containing 50 c.c.s.

of Czapek-Dox medium were each inoculated with a 5 mm. square of plate culture. The inocula were cut from regions 5 mm. behind the edges of three to four week old plate cultures of the fungi growing on malt agar. After inoculation the flasks were constantly shaken to aerate the medium and were kept in a water bath at 20°C.

After fourteen days the dry weights of the mycelium which had grown in each flask was determined. All were less than 0.200 grams. (Table 6) and so were too small for comparisons to be made with any accuracy. However, as the growth of these fungi on Czapek agar is also poor, it is possible that, if some other liquid medium had been used, a more vigorous growth might have been obtained.

Table 6.

DRY WEIGHTS OF FOURTEEN DAY OLD CZAPEK-DOX CULTURES.

Species Replication	Dry wt. in grams.	
	Culture a.	Culture b.
Tricladium gracile	0.00	-
Tricladium splendens	0.00	0.00
Tetracladium marchalianum	0.16	0.18
Articulospora tetracladia	0.18	0.11

3. INVESTIGATION INTO THE EFFECT OF VARIATIONS IN TEMPERATURE AND MEDIUM ON GROWTH.

(a) METHOD OF INVESTIGATION AND ANALYSIS OF THE RESULTS.

An investigation was made into the effect of four media, malt agar, Czapek agar, plain agar and maize agar, and five temperatures, 5°, 15°, 20°, 25° and 30°C. on the growth of the five species Tricladium gracile,

Tricladium splendens, Tetracladium marchialanum, Articulospora tetracladia and Clavariopsis aquatica.

Inocula were obtained from three week old cultures grown on malt agar at 20°C. 3 mm. square inocula were cut from a ring 5 mm. behind the margin of the colony. The experimental plates were 10 cm. petri dishes containing 20 c.c.s. of medium. The nature of and preparation of the media is outlined in Appendix 1. An inoculum was placed in the centre of each plate upperside uppermost. The plates were then inverted and four diameters were marked on the bottom of each plate in such a way that they intersected at a point corresponding to the approximate centre of the inoculum. Three replicate plates of each fungus on each medium were placed at each temperature. The inoculum was measured after each plate had been inoculated and the diameters of the colony growing from it were measured on every second day for a period of twenty eight days.

Three methods of expressing growth increments for each time interval were considered:- (1) increments in diameter, (2) increments in area and (3) increments in area proportional to the area of the colony at the end of the preceeding time interval. It was found that the use of areas exaggerated differences in growth rate and that the use of an increment in area proportional to the area of the colony at the end of the previous time interval eliminated certain marked differences in growth rate. Increments in diameter reduce differences only slightly and were therefore selected as being the most satisfactory measure of growth.

The growth increments during the first six days were very variable and so the first six day period was omitted from the statistical analysis of the results. The increments in growth for the succeeding ten days seemed to be constant and so the ten day period from the

sixth day to the sixteenth day was selected for statistical analysis. The increments in diameter for the two day periods during this ten day period are tabulated in Table A, Appendix 2. Each value in this table is an average of three replicates each of which is itself an average of four diameters. The ten day increments in diameter for each replicate are tabulated in Table B, Appendix 2.

Two methods of statistical analysis were employed - analysis of variance and linear regression analysis. Examples of these two methods are given in the Appendix 3. An analysis of variance assumes that the variance within each block of values is constant for all blocks. However at $30^{\circ}C$. in fifty per cent of the sets of values all the numbers are zero and so the variance of these sets is zero and is therefore not equal to the variance in the other sets of numbers. Therefore in order to ensure the accuracy of the analysis, the values for $30^{\circ}C$. were omitted from the analysis of variance.

The following statistical analyses were carried out:

(i) Analysis of variance of Table A excluding $30^{\circ}C$.

This analysis shows whether or not the main effect of time and its interaction effects with temperature, species and medium on the two-day increments of growth are significant for the ten day period under consideration. The purpose of this analysis was to justify the application of the linear regression analyses.

(ii) Linear regression analyses of the sets of five values for each treatment.

The aim of these analyses together with the analysis of variance of Table A was to establish that the rate of growth for the two day periods showed no variation and thus to justify an analy-

sis of the total growth over the whole of the ten day period. (Table B).

(iii) Analysis of variance of Table B excluding 30°C.

This analysis was carried out to establish whether or not the replication main effect and its first and second order interactions are significant and having established that they can be disregarded to show whether or not the other main effects and interactions are significant.

(iv) Analysis of variance of Table B excluding 30°C. and Czapek agar.

This analysis was carried out because, in the analysis of variance including Czapek agar, the main effect of medium and the first order interaction between medium and species are rather higher than the variance ratios for the other main effects and first order interactions respectively and it was thought that the generally poor growth on Czapek agar might be largely responsible for this. In this analysis these two variance ratios drop considerably and thus the result of the analysis becomes much more homogeneous. Only this analysis of variance is therefore considered in detail.

(v) Analysis of variance of Table B including 30°C. and Czapek agar.

The residual error from this analysis was used in the calculation of the standard error of the difference between replication means used in the discussion of the results for 30°C. and Czapek agar.

The results of these analyses will now be discussed in more detail.

TABLE 8. TABLE OF TIME-MEDIUM INTERACTION TOTALS.

Time in days. / Medium.	2	4	6	8	10	Medium Totals.
Malt agar	56.6	60.6	61.9	63.1	64.7	306.9
Czapek agar	46.3	48.9	47.1	48.5	48.5	237.6
Plain agar	68.9	67.1	65.2	61.7	64.0	326.9
Maize agar	64.2	62.9	63.2	65.8	64.1	320.2
Time Totals	236.0	239.5	237.4	237.4	241.3	1191.6

Time-medium interaction totals in m.m.

TABLE 11. VARIANCE TABLE FOR ANALYSIS OF VARIANCE OF TABLE B EXCLUDING 30°C. (SUMS OF FOR REPLICATION AND ITS INTERACTIONS ARE INCLUDED IN ERROR).

ITEM		Sum of Squares.	Degrees of Freedom.	Mean Square.	Variance Ratio.	Probability
Main effects	Temperature	7067.98	3	2355.99	515.53	0.001
	Medium	2282.08	3	760.69	166.45	0.001
	Species	2616.29	4	654.07	143.12	0.001
1st order interaction	Temperature-Species	1183.31	12	98.61	21.58	0.001
	Temperature-Medium	770.62	9	85.62	18.74	0.001
	Species-Medium	2104.35	12	175.36	38.37	0.001
2nd order interaction	Temp.-Spp.-Med.	1525.73	36	42.38	9.27	0.001
Residual	Error	721.67	158	4.57		
		18272.73	237			

NOTE:- The total number of degrees of freedom and the error degrees of freedom have been reduced by 2 to take into account the two "estimated" values in table B (Appendix 2) which are included in this analysis.

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NOTE:- The total number of degrees of freedom and the error degrees of freedom have been reduced by 2 to take into account the two "estimated" values in table B (Appendix 2) which are included in this analysis.

(i) Analysis of variance of Table A excluding 30°C.

It will be seen from Table 7, the variance table for the analysis of variance of Table A (Appendix 2) excluding 30°C., that the main effect of time and the first and second order interactions involving time are not significant except for the interaction between time and medium which is significant only at the 0.05 probability level. The value for plain agar at the 8th time interval in the time-medium interaction table (Table 8) is relatively very low and this probably accounts for the slight significance of the time-medium interaction. As the main effect of time is not significant, and its interactions are almost entirely not significant a linear regression analysis of each set of two day increments for the ten day periods is justified.

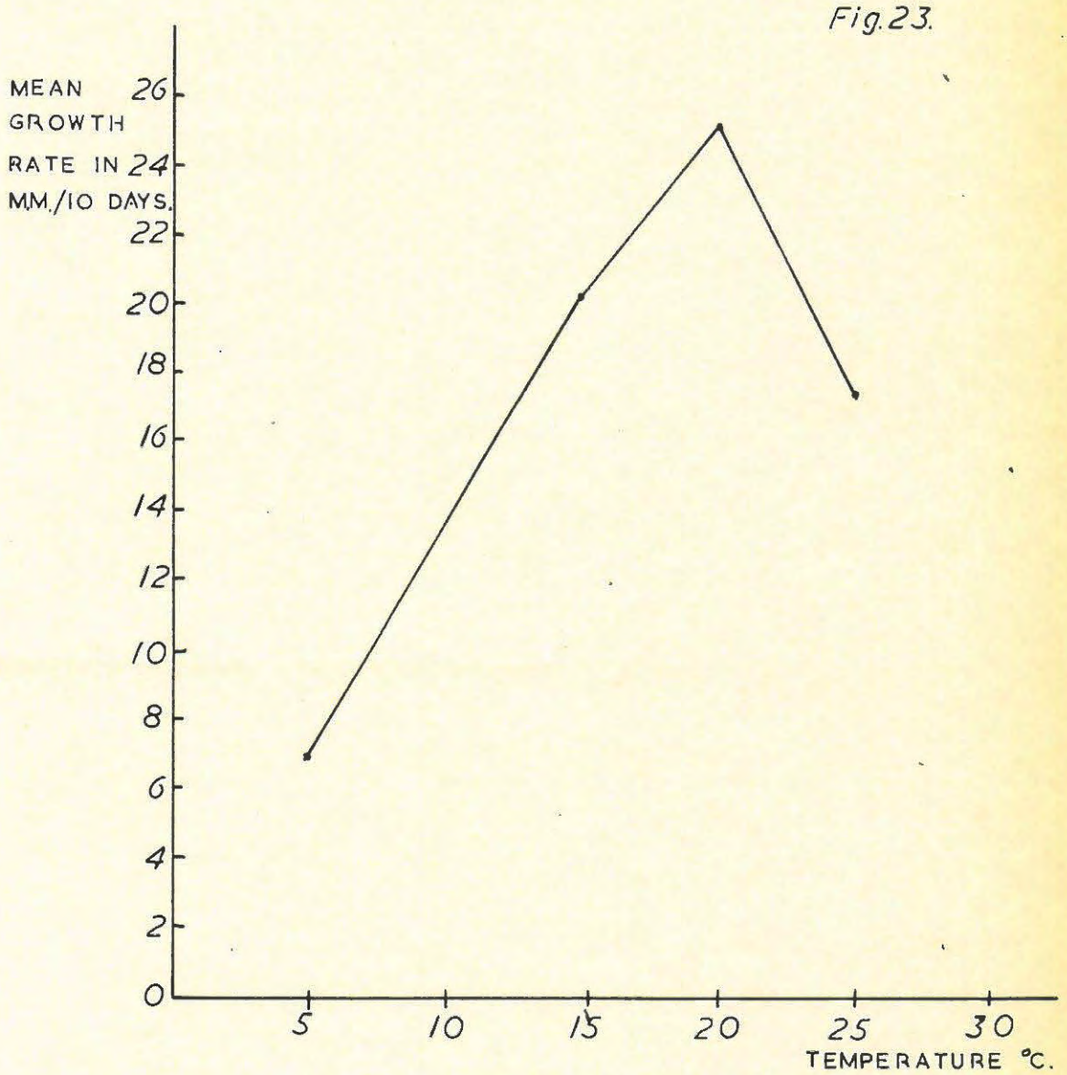
(ii) Linear Regression Analyses.

Only the three "t" values for these linear regression analyses indicated on Table 9 can be considered to be significant. It is therefore considered that there are no significant trends in the growth rate during the ten day period. The increments in diameter for this period therefore represent comparable units of growth.

(iii) Analysis of variance of Table B excluding 30°C.

The results of an analysis of variance of the increments in diameter for the ten day period tabulated in Table B (Appendix 2), excluding the values for 30°C. are tabulated in the variance table, Table 10. It is clear that the main effect of replication and all the first and second order interactions involving replication are not significant. The main effects of temperature, medium and species and the first and second order interactions between temperature, medium and species are all highly significant (Table 11). This shows that the curves for growth rate against temperature are different for each medium - species

Fig.23.



SCALE 1 c.m. = 2 m.m. on vertical axis.
2 cm = 5 °C. on horizontal axis.

S.E. of diff. bet. means = 0.45.

General curve for temperature for the ten day period.
(data from Table 14 A.)

combination. The feature dominating the whole analysis is the very small error variance. The standard error for the average of the three replications which determines a point on the growth rate against temperature curves is as small as 1.2. In other words the curves are very accurately determined and even small differences can be proved to be statistically significant. The very large species-medium interaction is explained by the fact that Articulospora tetracladium and Tetracladium marchialanum grow on Czapek agar as well as they do on the other media, whereas the growth of the other species on Czapek agar is very poor (Table 12).

(iv) Analysis of variance of Table B excluding 30°C. and Czapek agar.

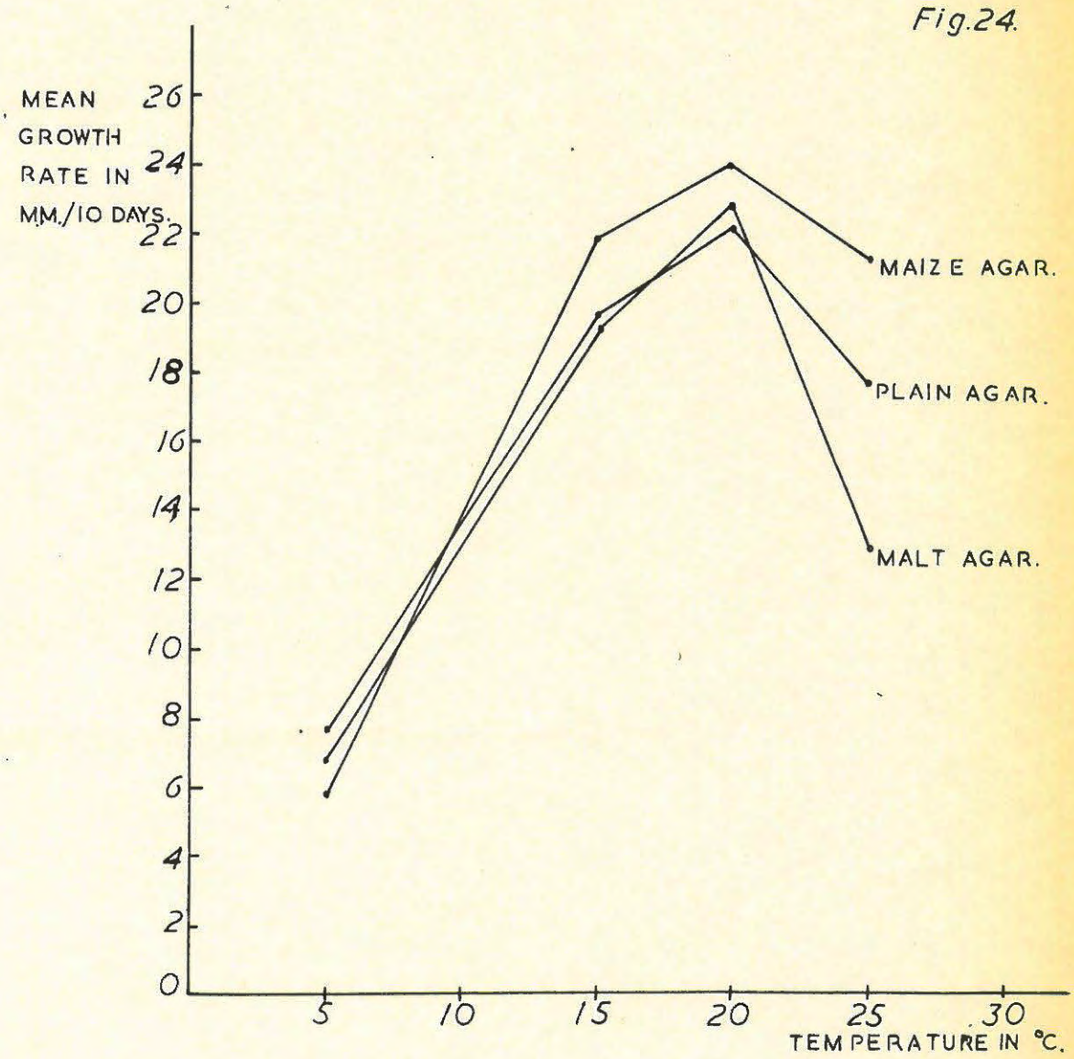
To test this conclusion an analysis of variance was made of Table B (Appendix 2) excluding the values for 30°C. and for Czapek agar. In this analysis, Table 13, the species-medium interaction variance ratio dropped to the level of those of the other first order interactions and the variance ratio for the main effect of medium was also considerably reduced. Thus excluding Czapek agar makes the results more homogeneous and this analysis will therefore be discussed in detail.

The overall effect of temperature is clearly apparent from Table 14, A and from Figure 23. With increasing temperatures from 5° to 20° C. there is a marked increase in growth rate but with a further 5° increase in temperature the growth rate decreases.

From Table 14, A it is clear that the general growth rate is significantly different on the three media, malt agar, plain agar and maize agar. The growth rate is highest on maize agar and lowest on malt agar.

It is apparent from Table 14, A that the general growth rates for the species are all significantly different

Fig.24.

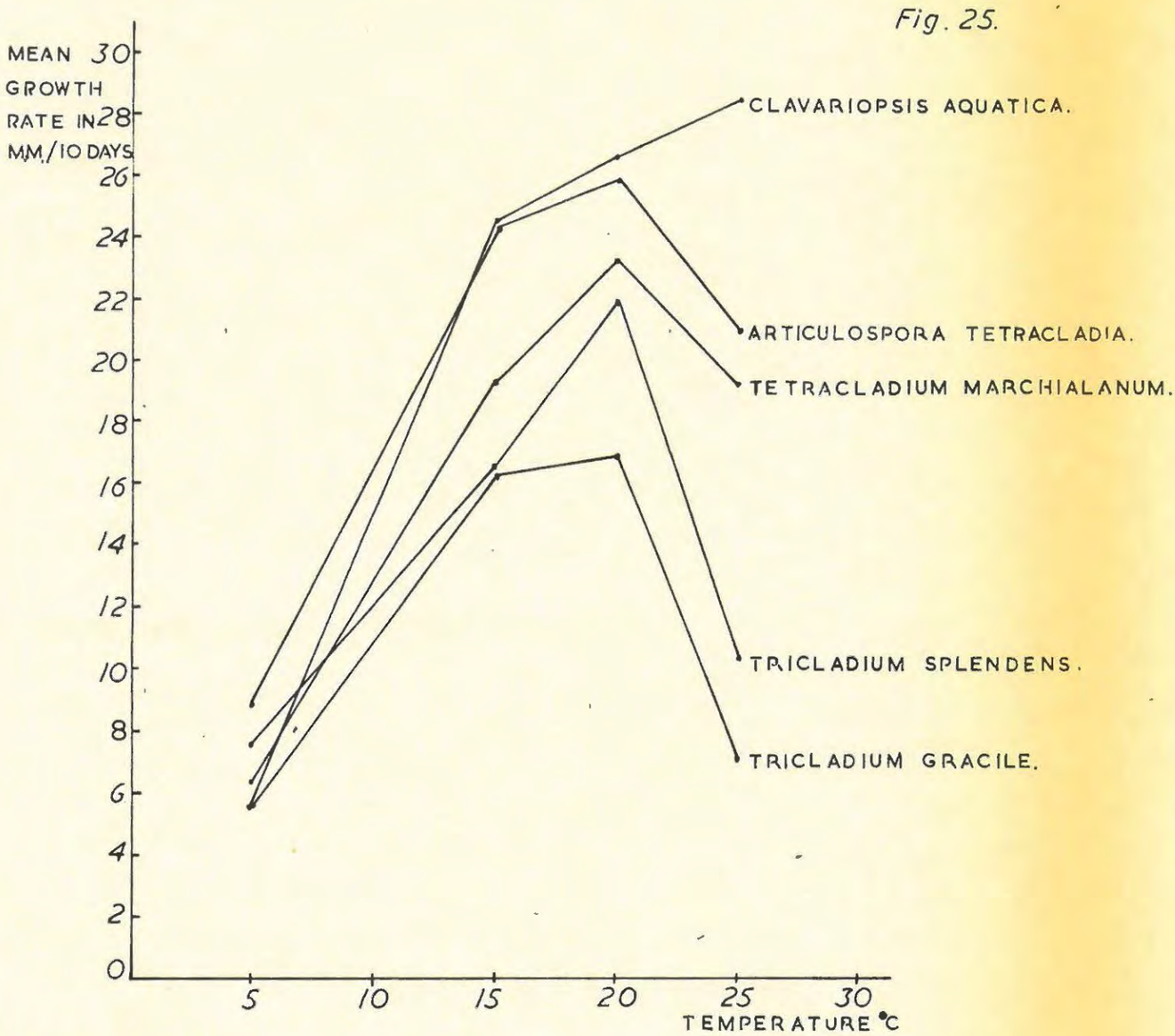


SCALE 1c.m.= 2m.m. on vertical axis.
2c.m.= 5 °C. on horizontal axis.

S.E. of diff. bet. means = 0.77.

Temperature–medium interaction curves for the ten day period. (data from Table 14 C.)

Fig. 25.



SCALE 1c.m. = 2mm. on vertical axis.

2c.m. = 5 °C on horizontal axis.

S.E. of diff. bet. means = 1·00

Species-temperature interaction curves for the ten day period.

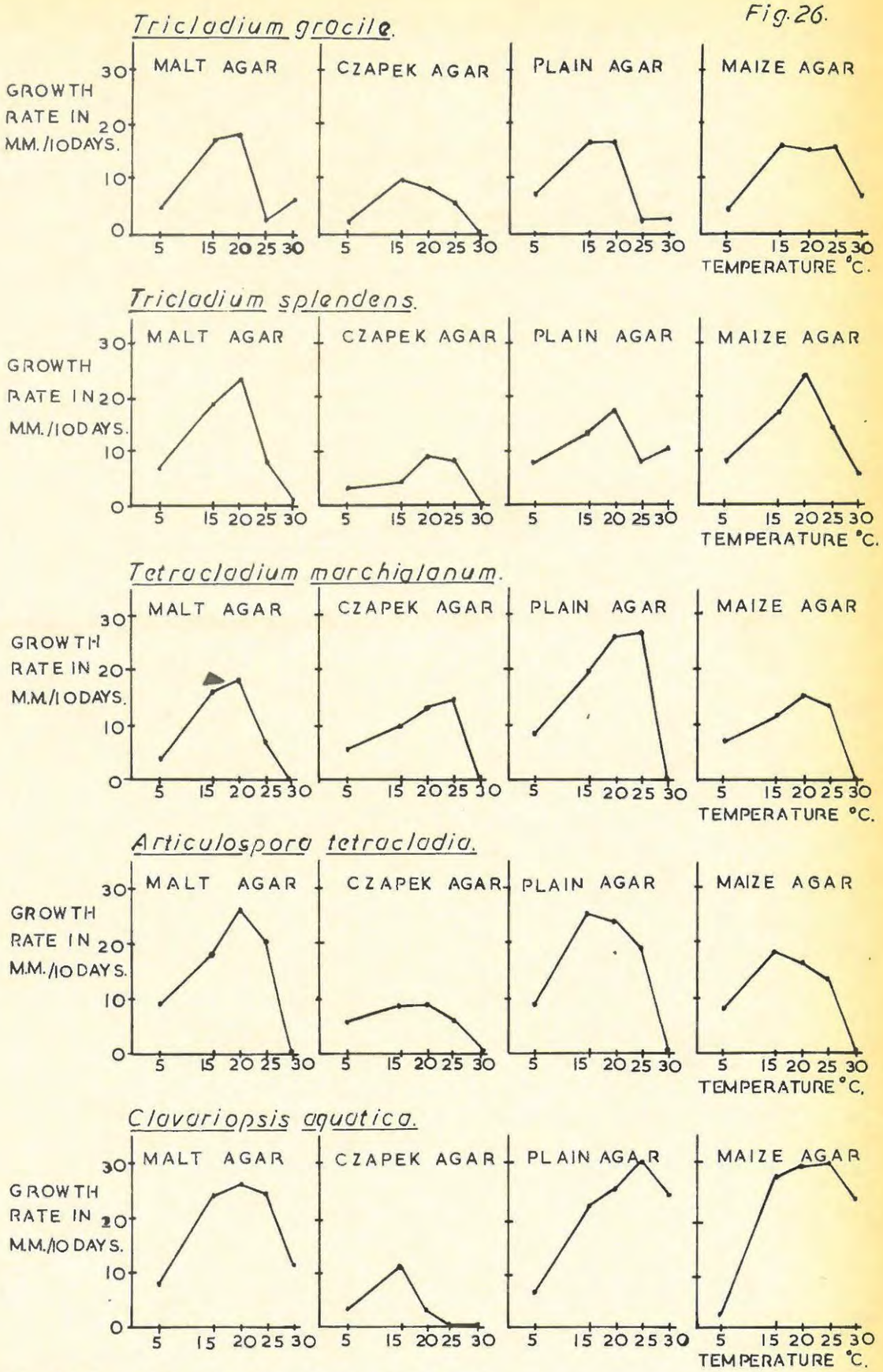
(data from Table 14 B.)

from each other and can be arranged in an ascending series Tricladium gracile, Tricladium splendens, Tetracladium marchialanum, Articulospora tetracladia and Clavariopsis aquatica.

The maize agar, plain agar and malt agar curves, Figure 24, for growth rate plotted against temperature are almost identical for temperatures up to 20°C. but at 25°C. they become widely divergent. At 25°C. the growth rate on plain agar is greater than that on malt agar, but smaller than that on maize. The highly significant medium main effect is probably accounted for by the divergence between the growth rates on the three media at 25°C. This requires further investigation.

The differences between the temperature curves for the five species (Fig. 25) become increasingly more marked with increasing temperatures. At 5°C, none of the species is significantly different from all the others. At 15°C. the growth rate of Tetracladium marchialanum is significantly different from those of the other four species which are grouped in pairs, one pair, Clavariopsis aquatica and Articulospora etetracladia having a larger growth rate, and the other pair, Tricladium splendens and Tricladium gracile, having a smaller growth rate. The growth rate of Tricladium splendens rises to the same level as that of Tetracladium marchialanum at 20°C. and so Tricladium gracile is then significantly different from the other four species. Tricladium splendens and Tetracladium marchialanum are the only two species in which the growth rate at 20°C. is significantly greater than that at 15°C. At 25°C. the difference is most striking. Clavariopsis aquatica, Tricladium splendens and Tricladium gracile are all significantly different from each other and from the other two species which are not significantly different. Clavariopsis aquatica further differs from all the other

Fig.26.



SCALE 1c.m. = 10m.m. on vertical axis.

1c.m. = 10°C. on horizontal axis.

S.E. of diff. bet. means = 1.72.

Growth rate plotted against temperature for each species-medium combination. (data from Table 15)

species in that its growth rate is higher at 25°C. than at 20°C., whereas in all the other species the growth rate is lower at 25°C. than at 20°C.

From a consideration of the species-medium interaction table (Table 14, D) it appears that Tetracladium marchialanum is probably largely responsible for the species-medium interaction being significant. In Table 14, D the species are arranged in order of their general growth rates from the lowest to the highest. On maize agar the growth rates of all the species and on malt and plain agar the growth rates of all the species except Tetracladium marchialanum conform to this general pattern. The growth rate of Tetracladium marchialanum on malt agar is as low as that of the slowest growing species and on plain agar is as high as that of the most rapidly growing species.

Summing up the results of the analysis excluding 30°C. and Czapek agar it can be said that there are clear overall effects of temperature and species but that the effect of medium is only apparent at 25°C. The species behave differently with respect to temperature, the differences being most marked at 25°C. The fall off in growth rate with a reduction in temperature from 20°C. is much more marked in some species than in others. The species react to medium in much the same way, except for Tetracladium marchialanum, the growth rate of which is significantly much lower on malt agar than on the other two media. This markedly low growth rate on malt agar seems to be contributed to by the low value at 25°C.

(v) The effects of Czapek agar and 30°C. are clearly apparent from a consideration of Table 15 and the temperature curves for each species on each medium (Fig. 26). Clearly the rate of growth of Tricladium gracile, Tricladium splendens and Clavariopsis aquatica

is much lower on Czapek agar than on the other three media but the rate of growth of Tetracladium marchialanum and Articulospora tetracladia on Czapek agar is as high as that on the other media. Another striking feature of the growth rate on Czapek agar is that Clavariopsis aquatica, in which the growth rate on the other three media is greater at 25°C. than at 20°C., grows more rapidly at 15°C. than at 20°C. and 25°C., when it is grown on Czapek agar.

At 30°C. the growth rate is on the whole much lower than at 25°C. This is most marked in Tetracladium marchialanum and Articulospora tetracladia in which the growth rate falls to zero at 30°C. The interaction between medium and species at 30°C. differs from that at 25°C. This variation would, however, require confirmation.

To sum up the analyses of all the treatments it can be said that there are clear overall effects of temperature and species but that the effect of media other than Czapek agar is only apparent at 25°C. and 30°C. The general rate of growth increases with increases in temperature from 5° to 20°C. and drops with further increases in temperature up to 30°C. The species all have different general rates of growth and can be arranged in an ascending series Tricladium gracile, Tricladium splendens, Tetracladium marchialanum, Articulospora tetracladia and Clavariopsis aquatica. The general growth rate on Czapek agar is lower than on the other three media. The difference in growth rate on the other three media is only apparent at 25°C. and at 30°C. and the order of magnitude of growth varies between the two temperatures. There is a fall off in growth rate with a decrease or an increase in temperature from 20°C. in all species except Clavariopsis aquatica which on all media except Czapek agar grows more rapidly at 25°C.

than at 20°C. the fall off in the growth rate of this species occurs above 25°C. All the species except Tetracladium marchialanum react to all the media except Czapek agar in much the same way. On Czapek agar Tricladium gracile, Tricladium splendens and Clavariopsis aquatica grow much more slowly than on the other media and Articulospora tetracladia and Tetracladium marchialanum grow at much the same rate as they do on the other media.

(b) DISCUSSION.

In a discussion of the relative growth rates on the various media at the various temperatures it should be borne in mind that growth is expressed as an increment in diameter and therefore by a linear measurement and not by one of volume or weight. So that, for example, it appears from the results that plain agar is a more suitable medium than malt agar. However, from an examination of the gross characteristics of the fungi on these two media it is almost certain that, if the volume or weight of the colonies were to be measured, the compact colony on malt agar would have a larger yield than the sparse but spreading colony on plain agar.

Another important consideration is that in this investigation possible variations in humidity were disregarded.

It seems worth noting that in cases, where there was little or no growth at 30°C. during the period of twenty eight days, this treatment was not lethal, as, when the cultures were removed from this temperature and placed at 20°C.,the colonies started to grow.

(c) CONCLUSIONS.

There are marked differences in reaction to temperature and medium between species, as represented by these isolates. However, only one isolate of each species was used in the investigation and, as it is well known that different isolates of the same species of fungus may show considerable variations in growth characteristics, it would be necessary to investigate the reaction to variations in temperature and medium of several isolates of each species before attempting to characterise the species. Similarly, it would be necessary to investigate a larger number of species before any general statements could be made of the reaction to temperature and medium of this group of aquatic Hyphomycetes as a whole. However, it seems of value to draw tentative conclusions from the available experimental results.

(i) The general reaction to variations in temperature is an increase in growth rate with increasing temperatures from 5^{o} to 20^{o}C. and a fall in growth rate with further increases in temperature to 30^{o}C. The optimal temperature lies between 15^{o} and 25^{o}C. most probably at about 20^{o}C. This confirms the conclusion of Tubaki (1957) for thirteen species including Articulospora tetracladia grown on malt agar.

(ii) The general growth rates on the four media are all different. The growth rate is highest on maize agar, followed by plain agar and then malt agar and is lowest on Czapek agar. It is however highly probable that this order would be different, if growth rate were to be measured as a volume or a dry weight of mycelium rather than as an increment in diameter.

(iii) The growth rate of each species irrespective of medium and temperature is significantly

different from that of each of the other species. The order of the species starting with the slowest growing and ending with the most rapidly growing species is Tricladium gracile, Tricladium splendens, Tetracladium marchialanum, Articulospora tetracladia and Clavariopsis aquatica.

(iv) The growth rates on malt agar, plain agar and maize agar are equal at temperatures up to 20°C. but at 25° and 30°C. the growth rates are different on these three media but the order of magnitude varies between the two temperatures. The general growth rate on Czapek agar at all temperatures is lower than on the other media.

(v) The species grouping based on growth rate is different for each temperature. The best separation of the species is obtained at 25°C. at which temperature the growth rates of Clavariopsis aquatica, Tricladium splendens, and Tricladium gracile are all significantly different from each other and from those of the other two species which are not significantly different from each other. Tetracladium marchialanum can however be separated at 15°C. at which its growth rate is different from those of the other four species. The growth rate of Articulospora tetracladia is not significantly different from the other species at any of the temperatures investigated.

(vi) In all species except Clavariopsis aquatica the growth rate at 20°C. is greater than that at 25°C. In Clavariopsis aquatica growing on all media except Czapek agar the growth rate at 25°C. is greater than at 20°C. The optimal temperature

for growth is therefore higher in Clavariopsis aquatica than in the other species.

(vii) The most marked difference in the growth rates of the five species on malt agar, Czapek agar, plain agar and maize agar is that on Czapek agar. Tricladium gracile, Tricladium splendens and Clavariopsis aquatica all grow more slowly on Czapek agar than on the other media but the growth rate of Articulospora tetracladia and Tetracladium marchialanum is as high on Czapek agar as it is on the other media.

4. CULTURE ON TWIGS.

The perfect stage of these aquatic Hyphomycetes is known in only two species Flagellospora penicillioides (Ranzoni 1953) and Heliscus lugdunensis (Webster 1959), both of which are species of Nectria. As species of Nectria commonly inhabit dead twigs in the local indigenous forests and exotic plantations, it was thought that it would be of interest to grow certain isolates of aquatic Hyphomycetes on sterilised twigs in the hope of obtaining the perfect stages.

250 c.c. flasks containing 5-10 c.c. of malt agar prepared with stream water were inoculated with Tricladium gracile, Tricladium splendens, Tetracladium marchialanum and Articulospora tetracladia. In the case of Articulospora tetracladia some of the flasks were inoculated with two isolates. After the colonies had become established in the flasks a moist sterilised twig was inserted into each flask in such a way that one end was embedded in the fungal colony. Dead twigs of Populus serotina, Quercus robur and Rhus legati were used and these varied in thickness from 1-2 cm. in diameter. The mycelia soon became established on the twigs which were then transferred to boiling tubes containing about

2 cm. of sterile moist sand. The twigs were examined from time to time and the sand was occasionally moistened with sterile distilled water. After nine months no fruiting bodies of any sort had been produced by any of the fungi.

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APPENDIX 1.

a) Preparation of media.

b) Isolation in pure culture.

a) PREPARATION OF MEDIA.

The media were prepared by suspending the dry ingredients in cold distilled or stream water, as indicated, and heating until dissolved. Aliquots of the required size were sterilised in tubes or flasks at 120°C. for fifteen minutes.

Two per cent malt agar for isolation of the fungi was prepared from powdered gelidium agar, Gurr's powdered malt extract and either distilled water or stream water. In some cases rose bengal was added in the concentration 1:20,000 and streptomycin in the concentration 35 per c.c. The streptomycin was added after sterilisation prior to the pouring of the agar into petri dishes.

The following four solid media were used in determining growth characteristics:-

(i) Two per cent malt agar prepared from Gurr's agar, Gurr's powdered malt extract and distilled water.

(ii) Two per cent Czapek agar prepared from Difco dehydrated Czapek agar and distilled water.

(iii) Two per cent maize agar prepared from Difco dehydrated maize agar and distilled water.

(iv) Two per cent plain agar prepared from Gurr's dehydrated agar and distilled water.

One liquid medium, Czapek-Dox broth was used and was of the following composition: sucrose 30 gm., sodium nitrate 2 gm., Potassium phosphate (K_2HPO_4) 1 gm., magnesium sulphate 0.5 gm., potassium chloride 0.5 gm., ferrous sulphate 0.01 gm., and distilled water 1,000 c.c.

b) ISOLATION IN PURE CULTURE.

Pure cultures were obtained by spraying spores onto the surface of two per cent malt agar plates and by subsequent sub-culturing onto fresh plates of the same

medium. High concentrations of fungal spores were collected in pipettes from the bubbles which were formed at the surface of the water around the sides of jars of aerated water in which leaves inhabited by these fungi were kept. Malt agar made with stream water seemed to be more satisfactory than that made with distilled water. It was found that the rate of growth but not the number of bacterial colonies could be considerably reduced, if rose bengal in the concentration 1:20,000 and streptomycin in the concentration 35 per c.c. were added to the medium.

TABLE A. TABLE OF INCREMENTS IN DIAMETER IN M.M. FOR THE FIVE TWO DAY PERIODS FROM THE SIXTH TO THE SIXTEENTH DAY.

TEMPERATURE. °C.		5					15					20				
Species	Time / Medium	2	4	6	8	10	2	4	6	8	10	2	4	6	8	10
	Agar															
Tricladium cracile.	Malt	1.0	1.2	0.8	1.2	1.2	3.2	3.2	3.2	3.2	3.0	3.6	3.4	3.8	4.1	3.6
	Czapek	0.1	0.6	0.4	0.3	0.9	1.9	2.2	1.8	1.6	1.5	2.0	2.1	1.6	1.5	1.6
	Plain	0.7	2.4	1.0	1.4	1.6	2.8	2.3	2.3	2.5	2.6	3.5	3.2	3.2	3.0	3.2
	Maize	0.5	1.2	0.7	0.8	1.1	2.4	2.5	3.0	2.8	2.9	3.4	3.0	3.3	2.9	2.7
Tricladium splendens.	Malt	1.1	1.6	1.3	1.7	1.6	3.0	3.6	4.0	4.2	4.0	5.9	4.9	4.2	4.5	5.0
	Czapek	1.2	1.4	1.9	1.7	1.8	2.3	3.7	3.5	3.7	3.2	5.3	5.2	4.3	4.3	5.0
	Plain	1.1	1.5	1.9	1.7	1.3	3.2	2.5	2.4	2.0	2.6	4.7	3.5	3.2	2.6	3.2
	Maize	0.4	1.2	0.7	0.7	0.3	1.0	0.9	0.7	1.0	1.0	2.0	1.3	2.4	2.1	1.6
Tetracladium marchalianum.	Malt	1.3	1.2	0.8	0.5	1.1	3.2	3.6	3.5	2.7	3.3	3.4	3.3	3.7	3.2	3.7
	Czapek	1.0	1.8	1.9	1.5	0.8	4.2	3.8	4.7	3.8	4.0	4.8	4.5	4.3	4.1	4.3
	Plain	2.0	1.9	1.8	1.1	1.3	3.6	4.5	4.1	3.4	3.8	6.3	5.1	5.2	4.9	4.7
	Maize	0.9	1.2	1.8	1.7	1.3	4.0	4.3	4.2	3.9	4.0	5.0	5.1	4.7	4.8	5.1
Articulospora tetracladia.	Malt	2.1	1.3	2.0	1.5	2.5	3.7	3.9	4.5	3.8	3.8	4.7	5.1	5.0	5.7	5.2
	Czapek	1.6	1.0	1.1	1.1	2.0	3.3	3.3	3.3	2.6	2.7	4.3	3.9	3.8	3.7	3.1
	Plain	2.7	1.5	1.4	2.1	1.9	4.3	4.6	4.5	4.2	4.1	4.8	5.1	5.2	4.5	5.0
	Maize	1.4	1.2	1.9	1.9	1.9	4.6	4.7	4.8	4.6	5.0	5.0	5.4	5.2	5.3	5.0
Clavariopsis aquatica.	Malt	0.9	1.5	1.5	1.9	2.2	4.5	4.5	4.9	4.6	5.2	4.5	5.4	5.0	5.5	5.5
	Czapek	0.7	0.8	0.8	0.8	0.1	0.9	1.2	1.9	2.8	3.2	0.3	0.4	0.3	1.2	0.6
	Plain	0.8	1.2	1.2	1.5	1.7	4.4	4.4	4.5	4.2	4.3	4.9	4.5	5.3	5.3	5.0
	Maize	0.6	0.5	0.5	1.0	0.7	7.4	4.4	4.7	4.8	4.8	6.1	5.4	5.4	5.9	6.1

Each value is an average of four diameters.

25					30				
2	4	6	8	10	2	4	6	8	10
0.4	0.4	0.4	1.0	0.8	1.1	1.2	1.2	1.2	1.8
1.0	0.9	0.9	1.2	1.5	0.0	0.0	0.0	0.0	0.0
0.5	0.2	0.4	0.6	0.9	0.8	1.3	0.9	0.6	0.6
2.4	3.0	3.1	3.2	2.8	1.0	1.6	1.8	1.6	1.6
1.4	1.2	1.8	2.2	1.9	0.2	0.2	0.4	0.6	0.6
2.5	2.7	2.7	3.4	2.9	1.0	0.8	1.0	1.9	1.3
2.0	1.4	1.7	1.8	1.4	2.1	2.7	2.3	1.8	2.0
2.0	1.6	1.4	1.9	1.8	0.1	0.2	0.1	0.0	0.1
0.8	0.9	1.7	1.8	1.7	0.0	0.0	0.0	0.0	0.0
4.5	5.3	4.7	4.5	4.4	0.0	0.0	0.0	0.0	0.0
5.9	5.8	5.9	5.0	5.0	0.0	0.0	0.0	0.0	0.0
5.5	5.3	4.0	4.7	4.8	0.0	0.0	0.0	0.0	0.0
3.3	4.3	4.8	4.6	3.4	0.0	0.0	0.0	0.0	0.0
3.9	4.1	3.2	3.0	4.9	0.0	0.0	0.0	0.0	0.0
3.9	4.8	3.9	3.6	3.3	0.0	0.0	0.0	0.0	0.0
3.6	4.6	4.9	5.1	4.9	0.0	0.0	0.0	0.0	0.0
4.6	5.9	5.0	5.2	6.0	1.6	2.1	3.1	2.7	3.3
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6.3	6.4	6.1	6.3	6.6	3.1	4.8	5.3	5.3	5.3
6.0	6.0	5.8	6.7	5.8	3.6	4.7	4.7	4.9	5.2

Appendix 2.

Table A – Table of increments in diameter in m.m. for the five two day periods from the sixth to the sixteenth day.

Table B – Table of increments in diameter in m.m. for the ten day period from the sixth to the sixteenth day.

Table C – Variance Table for Analysis of Variance of Table B.

TABLE B. TABLE OF INCREMENTS IN DIAMETER IN M.M. FOR THE TEN DAY PERIOD FROM THE SIXTH TO THE SIXTEENTH DAY.

Temperature °C.		5			15			20			25			30		
Species	Replication. / Medium	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
1. Tricladium gracile.	Malt Agar	5.0	5.0	5.0	16.5	16.5	18.0	19.0	17.5	20.0	2.0	4.0	2.0	4.5	8.5	(6.5)
	Czapek Agar	2.5	3.0	2.0	9.0	10.5	10.0	10.5	8.0	6.5	8.0	4.5	4.5	0.0	0.0	0.0
	Plain Agar	7.0	7.0	7.5	17.0	16.5	15.0	16.0	18.5	16.0	3.5	2.5	3.5	3.5	4.5	3.5
	Maize Agar	4.0	5.0	4.0	16.0	15.0	15.5	17.0	14.0	14.0	17.0	14.0	(15.5)	6.0	8.0	7.0
2. Tricladium splendens.	Malt Agar	7.5	7.5	8.0	18.0	22.5	17.5	24.5	24.0	23.0	8.5	8.5	8.5	1.5	1.0	2.0
	Czapek Agar	4.0	2.5	3.5	5.5	4.0	2.5	9.0	7.5	10.5	8.0	8.5	10.0	0.5	0.5	0.0
	Plain Agar	7.0	8.0	7.5	13.0	14.5	12.5	17.0	18.0	18.5	7.5	8.0	9.0	9.5	11.5	11.0
	Maize Agar	9.0	7.5	7.0	17.0	17.0	17.0	24.5	24.0	24.5	12.5	12.5	18.0	1.5	2.0	14.5
3. Tetracladium marchialanum.	Malt Agar	3.5	4.0	4.5	16.5	16.5	16.0	19.5	18.0	18.5	6.5	7.5	7.0	0.0	0.0	0.0
	Czapek Agar	5.5	7.5	4.5	20.0	20.0	20.0	22.5	23.0	24.0	26.0	22.0	26.0	0.0	0.0	0.0
	Plain Agar	8.0	9.5	8.0	26.0	17.0	18.0	24.0	30.0	24.5	21.5	22.5	38.0	0.0	0.0	0.0
	Maize Agar	7.0	6.5	6.5	21.0	22.0	20.5	24.5	25.5	25.0	24.5	23.0	22.0	0.0	0.0	0.0
4. Articulospora tetracladia.	Malt Agar	11.0	9.0	9.0	14.0	15.0	27.0	26.5	25.5	27.0	21.0	21.0	20.0	0.0	0.0	0.0
	Czapek Agar	2.0	8.0	7.5	18.0	19.0	18.5	20.0	18.5	18.0	19.5	18.5	10.0	0.0	0.0	0.0
	Plain Agar	8.0	9.0	10.0	27.5	27.5	22.0	25.5	23.0	24.5	21.5	22.0	13.5	0.0	0.0	0.0
	Maize Agar	9.0	7.0	8.5	28.5	28.0	29.5	25.0	27.0	28.5	23.0	24.0	23.5	0.0	0.0	0.0
5. Clavariopsis aquatica.	Malt Agar	7.0	7.5	8.0	23.5	24.0	24.0	25.0	26.5	26.5	23.5	24.5	26.5	8.0	14.5	(11.0)
	Czapek Agar	5.0	3.5	2.0	10.0	12.0	(11.0)	0.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Plain Agar	5.0	7.5	7.0	24.0	21.0	22.5	25.0	25.5	26.0	32.0	31.0	29.0	23.0	25.5	24.0
	Maize Agar	3.0	2.5	2.5	27.5	27.0	27.0	28.0	29.5	28.0	30.5	30.5	29.0	23.5	24.0	(24.0)

(no) value obtained by averaging the other two replicates in the set.

Each value is the average of 4 diameters.

TABLE C. VARIANCE TABLE FOR ANALYSIS OF VARIANCE OF TABLE B.

	Item.	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio.	Probability
Main effects.	Temperature	12226.43	4	3056.61	686.76	0.001
	Medium	2819.74	3	939.91	211.22	0.001
	Species	2331.69	4	582.92	130.99	0.001
1st order interaction	Temperature-Species	3221.70	16	201.36	45.25	0.001
	Temperature-Medium	811.30	12	67.61	15.19	0.001
	Species-Medium	2441.85	12	203.49	45.73	0.001
2nd order interaction	Temp.-Sp.-Med.	2107.57	48	43.91	9.87	0.001
Residual	Error	868.16	195	4.45		
TOTAL		26828.44	294			

Total degrees of freedom and error degrees of freedom are reduced by 5 to take into account the five "estimated" values indicated in Table B.

(4)

Appendix 3.

Statistical methods used in the analysis of the experimental data.

a) Analysis of variance.

b) Standard Errors.

c) Linear Regression Analysis.

STATISTICAL METHODS USED IN THE ANALYSIS OF THE EXPERIMENTAL DATA.

a) Analysis of variance. The analysis of variance of Table B excluding both 30°C and Czapek agar is given as an example of this method of analysis

TABLE OF SPECIES – TEMPERATURE INTERACTION TOTALS OF INCREMENTS IN DIAMETER FOR THE TEN DAY PERIOD.

Species \ Temperature °C.	5	15	20	25	Species Totals.
Tricladium gracile	49.5	146.5	152.0	64.0	412.0
Tricladium splendens	69.0	149.0	198.0	93.0	509.0
Tetracladium marchialanum	57.5	173.5	209.5	172.5	613.0
Articulospora tetracladia	80.5	219.0	232.5	189.5	721.5
Clavariopsis aquatica	50.0	220.5	240.0	256.5	767.0
Species totals	306.5	908.5	1032.0	775.5	3022.5

TABLE OF TEMPERATURE-MEDIUM INTERACTION TOTALS OF INCREMENTS IN DIAMETER FOR THE TEN DAY PERIOD.

Temperature °C. / Medium	5	15	20	25	Medium Totals.
Malt agar	101.5	285.5	341.0	191.0	919.0
Plain agar	116.0	294.0	332.0	265.0	1007.0
Maize agar	89.0	320.0	359.0	319.5	1096.5
Temperature totals	306.5	903.5	1032.0	775.5	3022.5

TABLE OF SPECIES-MEDIUM INTERACTION TOTALS OF INCREMENTS IN DIAMETER FOR THE TEN DAY PERIOD.

Species / Medium	Tricladium gracile	Tricladium splendens	Tetracladium marchialanum	Articulospora tetracladia	Clavariopsis aquatica	Medium Totals
Malt agar	130.5	178.0	138.0	226.0	246.5	919.0
Plain agar	130.0	140.5	247.0	234.0	255.5	1007.0
Maize agar	151.5	190.5	228.0	261.5	265.0	1096.5
Species totals	412.0	509.0	613.0	721.5	767.0	3022.5

$$\text{Correction Factor} = \frac{(3022.5)^2}{180} = \frac{9135506.25}{180}$$

$$= 50752.8125$$

Sums of Square for Temperature

$$= \frac{(306.5)^2 + (908.5)^2 + (1032.0)^2 + (775.5)^2}{45} - 50752.8125$$

$$= 57460.8611 - 50752.8125$$

$$= 6708.0486$$

Sum of Squares for Medium

$$= \frac{(919.0)^2 + (1007.0)^2 + (1096.5)^2}{60} - 50752.8125$$

$$= 51015.3708 - 50752.8125$$

$$= 262.5583$$

Sum of Squares for Species

$$= \frac{(412.0)^2 + (509.0)^2 + (613.0)^2 + (721.5)^2 + (767.0)^2}{36} - 50752.8125$$

$$= 53151.2569 - 50752.8125$$

$$= 2398.4444$$

Sum of Squares for Temperature - Species Interaction.

$$= \frac{551902.75}{9} - (50752.8125 + 6708.0486 + 2398.4444)$$

$$= 61322.5278 - 59859.3055$$

$$= 1463.2223$$

Sum of Squares for Temperature-Medium Interaction.

$$= \frac{872038.75}{15} - (50752.8125 + 6708.0486 + 262.5583)$$

$$= 58135.9167 - 57723.4194$$

$$= 412.4973$$

Sum of Squares for Species-Medium Interaction.

$$= \frac{647115.75}{12} - (50752.8125 + 262.5583 + 2398.4444)$$

$$= 53926.3125 - 53413.8152$$

$$= 512.4973$$

TABLE OF TEMPERATURE-SPECIES-MEDIUM INTERACTION TOTALS OF INCREMENTS DIAMETER FOR THE TEN DAY PERIOD.

Species \ Temperature. / Medium		5	15	20	25
Tricladium gracile	Malt agar	15.0	51.0	56.5	18.0
	Plain agar	21.5	48.5	50.5	9.5
	Maize agar	13.0	47.0	45.0	46.5
Tricladium splendens	Malt agar	23.0	54.0	71.5	29.5
	Plain agar	22.5	40.0	53.5	24.5
	Maize agar	23.5	51.0	73.0	43.0
Tetracladium marchial-anum	Malt agar	12.0	49.0	56.0	21.0
	Plain agar	25.5	61.0	78.5	82.0
	Maize agar	20.0	63.5	75.0	69.5
Articulospora tetra-cladia	Malt agar	29.0	56.0	79.0	62.0
	Plain agar	27.0	77.0	73.0	57.0
	Maize agar	24.5	86.0	80.5	70.5
Clavariopsis aquatica	Malt agar	22.5	71.5	78.0	74.5
	Plain agar	19.5	67.5	76.5	92.0
	Maize agar	3.0	81.5	85.5	90.0

Sum of squares for Temperature-Species-Medium Interaction.

$$= \frac{189291.75}{3} - (50752.8125 + 6708.0486 + 262.5583 + 2398.4444 + 1463.2223 + 412.4973 + 512.4973)$$

$$= 63097.2500 - 62510.0807$$

$$= 587.1693$$

VARIANCE TABLE.

ITEM		Sum of Squares.	Degrees of Freedom.	Mean Square.	Variance Ratio.	Probability
Main Effects.	Temperature	6708.05	3	2236.02	496.89	0.001
	Medium	262.56	2	131.28	29.17	0.001
	Species	2398.44	4	599.61	133.25	0.001
1st order interactions.	Temperature-Species	1463.22	12	121.94	27.10	0.001
	Temperature-Medium	412.50	6	68.75	15.28	0.001
	Species-Medium	512.50	8	64.06	14.24	0.001
2nd order interaction.	Temperature-Species-Medium	587.17	24	24.47	54.38	0.001
Residual	Error	535.00	119	4.50		
TOTAL		12879.44	178			

NOTE:- The total degrees of freedom and error degrees of freedom have been reduced by one to take into account the "estimated" value in table B.

b) Standard Errors used in the analysis.

(i) Standard Error of the difference between means.

S.E. of difference between means $= \sqrt{\frac{2 \text{ Error}}{x}}$ mm

where Error is obtained from the Variance Table

and x = number of values summed and averaged to obtain each mean value.

TABLE OF X VALUES FOR ANALYSIS EXCLUDING CZAPEK AGAR AND 30°C.

Means	X
Temperature	45
Medium	60
Species	36
Temperature-Species	9
Temperature-Medium	15
Species-Medium	12

FOR ANALYSIS INCLUDING CZAPEK AGAR AND 30°C.

S.E. of difference between replication means x = 3

S.E. of difference between medium means for each species x = 15

(ii) Standard Error of the difference between totals.

S.E. of difference between totals $= \sqrt{\frac{\text{Error}}{x}}$ mm

Where Error is obtained from the Variance Table and x = number of values summed to obtain each total.

For S.E. of difference between replication totals excluding 30°C. x = 3.

c) Linear Regression Analysis.

(i) Equation of the line

$$X = Mx + bxy \cdot (y - My)$$

Where X = average value of the x variates that may be expected when the value of the y variable is fixed at y.

Mx = mean of x

My = mean of y

$$bxy = \frac{SPxy}{SSy}$$

$$SPxy = \text{Sum product of } xy = xy - \frac{x \; x \; y}{n}$$

$$SSy = \text{Sum of square of } y = y^2 - \frac{(y)^2}{n}$$

Where n = number of x or y values

(ii) Test of significance of regression function.

S.E. bxy = Standard error of bxy =

$$\sqrt{\frac{SSx - \frac{(SPxy)^2}{SSy}}{(n-2) \quad (SSy)}}$$

where $SSx = \text{Sum of squares of } x = x^2 - \frac{(x)^2}{n}$

$$t = \frac{bxy}{SE\ bxy}$$

Reference to the t table for n-2 degrees of freedom will show whether this calculated "t" is greater or less than "t" for a given probability. In these linear regressions n-2 = 3. $t_3 = 3.182$ at the 0.05 probability level. "t" values smaller than this were regarded as not significant.

(iii) Example of Linear Regression Analysis.

The linear regression analysis for Tricladium splendens on malt agar at 5°C. is given as an example of this type of analysis.

x = growth increment in mm.

y = time increment in days.

	x	y	x^2	y^2	xy
	1.1	2	1.21	4	2.2
	1.6	4	2.56	16	6.4
	1.3	6	1.69	36	7.8
	1.7	8	2.89	64	13.6
	1.6	10	2.56	100	16.0
Totals	7.3	30	10.91	220	46.0

Sum of Squares for $x = 10.91 - \frac{7.3^2}{5} = 10.91 - 10.658$

$= 0.252$

Sum of Squares for $y = 220 - \frac{30^2}{5} = 220 - 180$

$= 40.$

Sum of the Product of xy =

$$46.0 - \frac{7.3 \times 30}{5} = 46.0 - 43.8$$

$$= 2.2$$

$$bxy = \frac{2.2}{40} = 0.055$$

Standard Error of $bxy = \sqrt{\frac{0.252 - \frac{2.2^2}{40}}{120}} = 0.0332$ mm.

$$t = \frac{bxy}{S.E.bxy} = \frac{0.055}{0.0332} = 1.6566$$

This value of "t" is smaller than 3.182 and there is therefore no significant trend in the x values.

TABLE 5. pH VALUES OF WATER FROM VARIOUS STREAMS DURING THE YEAR APRIL 1960 - APRIL 1961.

Site.	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
HOGSBACK, Wolf Ridge.	7.49											
Poplar 1			7.99	7.0	8.3					7.25		
Oak	7.35		7.6	6.0	7.7	7.1						
Poplar 2			7.2	6.8	6.6		7.1					
Natural Forest	7.3		7.6	7.1	7.0					7.2		
Mixed Plantation 1.	7.4			7.15						7.25		
2.	7.4		7.7	7.35						7.25		
GRAHAMSTOWN.												
Featherstone Kloof					7.15	7.1						
Paradise Kloof						7.2 7.3	6.75 7.3	6.5 7.9	6.5 7.15		6.8 6.85	
Howiesons Poort	7.4 7.95					6.6 7.1	6.5 6.65					
Rabbit Bush				6.35								
Belmont Valley					7.2	7.3						
TYUMIE RIVER			7.6							7.4		
PORT ELIZABETH												
Willow						7.55						
Poplar						8.15						

TABLE 7.

VARIANCE TABLE FOR THE ANALYSIS OF VARIANCE OF TABLE A

(APPENDIX 2) EXCLUDING 30°C.

	Item.	Sum of Squares.	Degrees of Freedom.	Mean Squares.	Variance Ratio.	Probability.
Main effects.	Temperature	42117.74	3	14039.25	902.27	0.001
	Medium	5055.38	3	1685.13	108.30	0.001
	Species	18269.31	4	4567.33	29.35	0.001
	Time	21.69	4	5.42	0.3483	not significant.
1st order interactions	Temp.-Spp.	7893.71	12	658.31	42.31	0.001
	Temp.-Med.	4003.34	9	444.82	28.59	0.001
	Spp.-Med.	23879.47	12	1989.96	127.89	0.001
	Temp.-Time	326.54	12	27.21	1.75	not significant.
	Spp.-Time	401.49	16	25.09	1.61	" "
	Medium-Time	371.90	12	30.99	1.99	0.05
2nd order interactions	Temp.-Spp.-Med.	10365.01	36	2879.17	185.04	0.001
	Temp.-Spp.-Time	977.89	48	20.37	1.31	not significant.
	Temp.-Medium - Time	438.58	36	12.18	0.7828	" "
	Medium-Spp.-Time	922.13	48	19.21	1.23	" "
3rd order interaction	Error	2240.18	144	15.56		
	TOTAL	117290.36	399			

TABLE 9. TABLE OF "t" VALUES FROM LINEAR REGRESSION ANALYSES OF EACH SET OF TWO DAY INCREMENTS IN DIAMETER FOR THE TEN DAY PERIODS IN TABLE A (APPENDIX 2).

Species. \ Temp °C. / Medium.		5	15	20	25	30
Tricladium gracile.	Malt agar	0.22	1.73	0.80	2.18	2.18
	Czapek agar	1.48	1.63	2.08	2.37	0.00
	Plain agar	0.03	1.26	1.73	1.87	1.31
	Maize agar	0.85	2.28	2.52	1.00	0.04
2. Tricladium splendens.	Malt agar	1.66	2.91	1.11	2.24	1.64
	Czapek agar	2.42	0.61	0.95	1.65	1.39
	Plain agar	0.54	1.36	2.27	0.04	1.57
	Maize agar	0.29	0.02	0.00	0.12	0.86
3. Tetracladium marchialanum.	Malt agar	1.09	0.58	0.00	3.16	0.00
	Czapek agar	0.41	0.30	0.84	0.81	0.00
	Plain agar	1.09	0.46	2.96	2.95	0.00
	Maize agar	0.61	0.71	0.15	1.11	0.00
4. Articulospora tetracladia.	Malt agar	0.60	0.00	1.20	0.20	0.00
	Czapek agar	0.57	2.68	(4.90)	0.33	0.00
	Plain agar	0.55	3.00	0.20	1.59	0.00
	Maize agar	0.86	1.53	0.16	2.48	0.00
5. Clavariopsis aquatica.	Malt agar	0.03	2.26	2.08	0.94	2.86
	Czapek agar	1.38	(3.27)	1.25	0.00	0.00
	Plain agar	2.19	1.16	0.93	0.84	2.42
	Maize agar	0.98	1.36	0.40	0.22	(3.39)

"t" = 3.182

"p" = 0.05

significant "t" values are ringed

TABLE 10.

VARIANCE TABLE FOR ANALYSIS OF VARIANCE OF TABLE B

EXCLUDING 30°C.

	Item.	Sum of Squares.	Degrees of Freedom.	Mean Squares.	Variance Ratios.	Probability
Main effects	Temperature	7067.98	3	2355.99	443.69	0.001
	Medium	2282.61	3	760.87	143.29	0.001
	Species	2616.29	4	654.07	123.18	0.001
	Replication	2.15	2	1.08	0.20	not significant
1st order interactions	Temperature-Species	1163.31	12	96.61	18.57	0.001
	Temperature-Medium	770.62	9	85.62	16.12	0.001
	Species-Medium	2104.55	12	175.38	33.03	0.001
	Medium-Replication	15.71	6	2.62	0.49	not significant
	Species-Replication	12.98	8	1.62	0.31	not significant
	Temperature-Replication	2.22	6	0.37	0.07	not significant
2nd order interactions	Temp.-Spp.-Medium	1525.73	36	42.38	7.98	0.001
	Temp.-Spp.-Rep.	175.56	24	7.32	1.38	not significant
	Temp.-Med.-Rep.	80.86	18	4.49	0.84	not significant
	Spp.-Med.-Rep.	60.20	24	2.51	0.47	not significant
Residual	Error	371.99	70	5.31		
		18272.73	237			

NOTE: The total number of degrees of freedom and the degrees of freedom for Error are reduced by 2 to take into account the two (estimated) values in Table B (Appendix 2) which are included in this analysis.

TABLE 12.

TABLE OF SPECIES-MEDIUM INTERACTION TOTALS EXCLUDING 30°C. FOR THE TEN DAY PERIOD.

Species / Medium.	Tricladium gracile.	Tricladium splendens.	Tetracladium marchialanum.	Articulospora tetracladia.	Clavariopsis aquatica.	Medium Totals.
Malt agar.	130.5	175.0	138.0	226.0	246.5	919.0
Czapek agar.	79.0	75.5	221.0	177.0	52.5	605.5
Plain agar.	130.0	140.0	247.0	240.0	255.5	1007.0
Maize agar.	151.5	190.5	228.0	261.5	265.5	1096.5
SPECIES TOTALS	491.0	584.0	834.0	899.0	819.5	3628.0

Totals in m.m.

TABLE 13. VARIANCE TABLE FOR ANALYSIS OF VARIANCE OF TABLE B (APPENDIX 2) EXCLUDING BOTH 30°C. AND CZAPEK AGAR.

	Item.	Sum of Squares.	Degrees of Freedom.	Mean Squares.	Variance Ratio.	Probability.
Main effects.	Temperature	6708.05	3	2236.02	496.89	0.001
	Medium	262.56	2	131.28	29.17	0.001
	Species	2393.48	4	598.37	133.25	0.001
	Temp.-Spp.	1463.22	12	121.94	27.10	0.001
1st order interactions	Temp.-Medium	412.50	6	68.75	15.28	0.001
	Spp.-Medium	512.50	8	64.24	14.24	0.001
2nd order interaction	Temp.-Spp.-Medium	587.17	24	24.47	54.38	0.001
Residual	Error	535.00	119	4.50		
	TOTAL	12879.44	178			

NOTE: The total degrees of freedom and the error degrees of freedom have been reduced by one to take into account the "estimated" value in Table B (Appendix 2) which is included in this analysis.

TABLE 14 A. TABLE OF MAIN EFFECT MEAN GROWTH INCREMENTS EXCLUDING 30°C AND CZAPEK AGAR FOR THE TEN DAY PERIOD.

Species	Tricladium gracile	Tricladium splendens	Tetracladium marchialanum	Articulospora tetracladia	Clavariopsis aquatica	S.E. of diff. between means
Means	11.44	14.14	17.03	20.04	21.31	0.50
Temp. °C.	5°	15°	20°	25°		S.E. of diff. between means
Means	6.81	20.19	25.16	17.23		0.45
Medium	Malt agar	Plain agar	Maize agar			S.E. of diff. between means
Means	15.32	16.78	18.28			0.39

Means in m.m.

TABLE 14 B. TABLE OF TEMPERATURE-SPECIES INTERACTION MEANS EXCLUDING 30°C AND CZAPEK AGAR FOR THE TEN DAY PERIOD.

Species \ Temp. °C	5	15	20	25
Tricladium gracile	5.50	16.28	16.89	7.11
Tricladium splendens	7.67	16.56	22.00	10.33
Tetracladium marchialanum	6.39	19.28	23.28	19.17
Articulospora tetracladia	8.94	24.33	25.83	21.06
Clavariopsis aquatica	5.56	24.50	26.67	21.31

Means in m.m. S.E. of diff. between Temp.-Species means 0.99

TABLE 14 C. TABLE OF TEMPERATURE-MEDIUM INTERACTION MEANS EXCLUDING 30°C AND CZAPEK AGAR FOR THE TEN DAY PERIOD.

Medium \ Temp. °C	5	15	20	25
Malt agar	6.77	19.03	22.73	12.73
Plain agar	7.73	19.60	22.13	17.67
Maize agar	5.93	21.93	23.93	21.30

means in m.m. S.E. of diff. between Temperature-medium means 0.77

TABLE 14 D. TABLE OF SPECIES-MEDIUM INTERACTION MEANS EXCLUDING 30° AND CZAPEK AGAR FOR THE TEN DAY PERIOD

Medium \ Species	Tricladium gracile	Tricladium splendens	Tetracladium marchialanum	Articulospora tetracladia	Clavariopsis aquatica.
Malt agar	10.83	14.83	11.50	18.83	20.54
Plain agar	10.83	11.71	20.58	19.50	21.29
Maize agar	12.63	15.83	19.00	21.79	22.08

means in m.m. S.E. of diff. between Species-Medium Means 0.87

TABLE 15. TABLE OF MEAN GROWTH INCREMENTS FOR TEMPERATURE-MEDIUM-SPECIES INTERACTION FOR THE TEN DAY PERIOD FROM TABLE B, APPENDIX 2.

Species \ Temp / Medium.	5	15	20	25	30	Medium Means for Each Species.
Tricladium gracile	Malt agar	5.0	17.0	13.8	2.7	6.5	50.0
	Czapek agar	1.8	9.8	8.3	5.7	0.0	26.3
	Plain agar	7.2	16.2	16.8	3.2	3.8	47.2
	Maize agar	4.3	15.7	15.0	15.5	7.0	57.5
Tricladium splendens	Malt agar	7.7	19.3	23.8	8.5	1.5	60.8
	Czapek agar	3.3	4.0	9.0	8.8	0.3	25.5
	Plain agar	7.5	13.3	17.8	8.2	10.7	57.5
	Maize agar	7.8	17.0	20.3	14.3	6.0	69.5
Tetracladium marchialanum	Malt agar	4.0	16.3	18.7	7.0	0.0	46.0
	Czapek agar	5.8	20.0	23.2	24.7	0.0	73.7
	Plain agar	8.5	20.3	26.2	27.3	0.0	82.3
	Maize agar	6.7	21.2	25.0	23.2	0.0	76.0
Articulospora tetracladia	Malt agar	9.7	18.7	26.3	20.7	0.0	75.3
	Czapek agar	5.8	13.5	15.8	16.0	0.0	59.2
	Plain agar	9.0	25.7	24.3	19.0	0.0	78.0
	Maize agar	8.2	28.7	26.8	23.5	0.0	87.2
Clavariopsis aquatica	Malt agar	7.5	23.8	26.0	24.8	11.2	93.3
	Czapek agar	3.5	11.0	3.0	0.0	0.0	17.5
	Plain agar	6.5	22.5	25.5	30.7	24.2	109.3
	Maize agar	2.7	27.2	28.5	30.0	23.8	112.2
						S.E. diff = 0.77	

S.E. of difference between means = 1.72 growth rates in m.m.

(Variance table for analysis of variance of Table B, Appendix 2, including Czapek agar and 30°C is given in Appendix 2, Table C.)