On aquatic hyphomycetes: a literature review and field investigation in lotic waters around Durham City

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ON AQUATIC HYPHOMYCETES

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ABSTRACT

This dissertation contains a review of much of the recent literature on the nature and ecology of those imperfect fungi known as aquatic Hyphomycetes, and an account of a field investigation of these fungi in flowing waters in, and around Durham City.

The review discusses the fungi themselves: their morphology, physiology and ecology. Their particular role as decomposers of deciduous leaves in lotic freshwater is highlighted through a discussion of decomposition in aquatic habitats, the role of fungi in the initial breakdown of plant material, and the importance of fungi as intermediates in the further breakdown of plant detritus. Field and laboratory methods useful in the study of fungal ecology are discussed.

The field investigation, carried out in late summer and early autumn, is of the flora and spora of 7 lotic sites of differing character, including a main river; certain of its tributaries, and a woodland stream. A combination of complimentary methods is used to build up a picture of the floras and spora. Filtration of water and examination of foam provide information on aquatic Hyphomycete spores whilst the observation of submerged leaf material (Acer pseudoplatanus), before and after incubation, provides information on impacted spores and growing fungi.

The floras and sporas recorded for the different sites, on the different dates, and by the different methods are compared and contrasted.

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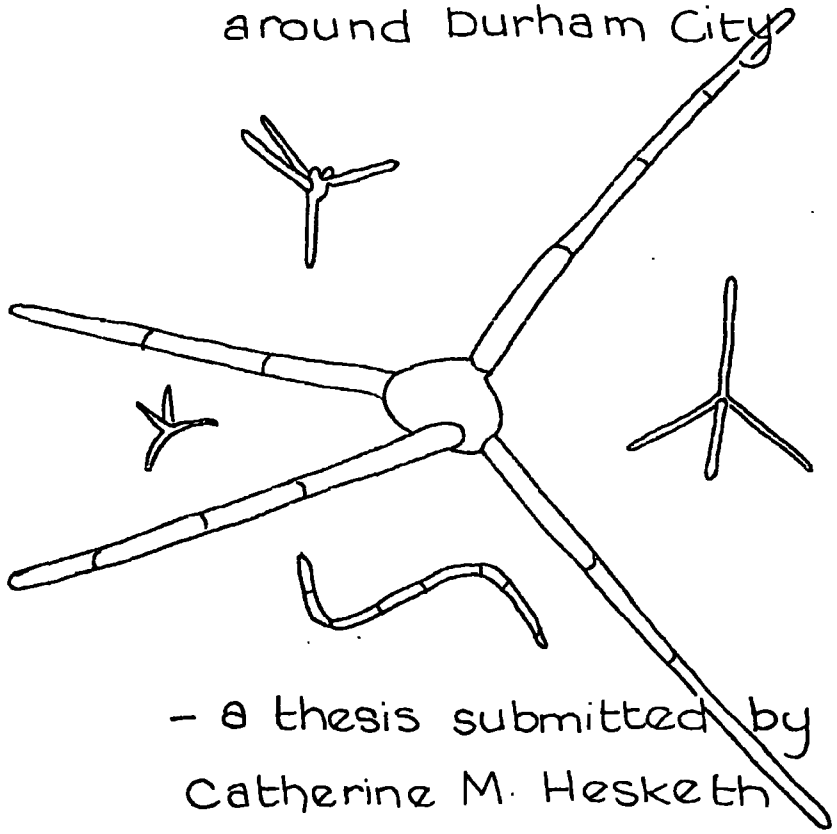

– a thesis submitted by
Catherine M. Hesketh
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for the degree of Master of Science in Ecology

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To

Leona, Bill and Geoffrey

My own suspicion is that the universe
is not only queerer than we suppose,
but queerer than we can suppose.

J B S Haldane

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CONTENTS

SPECIAL NOTE

A Summary Pattern illustrating the relationship between the diverse areas of study in the biology of aquatic Hyphomycetes can be found as a separate sheet at the back of the thesis. This may be useful in relating the various topics discussed throughout the thesis to the particular ecology of aquatic Hyphomycetes.

Certain large tables (e.g. Table 2.6 and data tables from Chapter 5) are also included loose at the back of the thesis rather than fixed in the body of the text.

In drawing up the various spore diagrams in Chapters 2 and 5, I would like to acknowledge extensive adaption of Ingold's clear line drawings of spores and sporophores. (references Ingold 1975a, 1975b).

1. INTRODUCTION

INTRODUCTION

1.1. Aims and Approach

The aim of this project was to gain an understanding of an ecologically interesting topic through the synthesis of previous work and analysis of information produced by study in the field. Also to develop the logistics and dexterity required to carry out practical work.

This particular study is of the ecological group of fungi known as aquatic Hyphomycetes. Equal weight has been given to both the literature and the field investigations. This was a practical possibility because of the necessity for a late summer sampling programme, and the modest volume of ecological work on these fungi in the literature. It was also particularly interesting to follow through the accumulation of ecological knowledge on these fascinating fungi by looking at the methods adopted in, and conclusions drawn from, studies with diverse approaches and aims.

A critical survey of relevant literature is always of great use when attempting to sort out which of many pertinent variables and interactions have been or could be investigated in the ecological study of a particular group of organisms. Such a survey helps to build up a basic biological background, helps in assessing the ecological significance of conclusions drawn, and is of use in deciding which combinations of practical approach are useful, what developments and adaptations of these are possible.

A critical approach seems to be a particularly useful tool in ecological work and understanding. In the study of ecology there can be many approaches to an understanding of the role of particular species or group, to the understanding of the function of a particular ecosystem. There are so many potentially important external and internal parameters

to consider and measure. What is the effect of temperature, pH or oxygen content of the environment, for instance; what is the biochemical basis of behaviour in animals or plants? Such a wide range of scientific techniques can be used in the study of even a single phenomenon.

Bias towards particular aspects of an area of ecological study are inevitable. These can be generated by scientific or economic restraint: lack of an accurate method of measuring an important environmental parameter, for instance; agricultural need has meant a stress on the study of pest organisms; whilst little work has been done on tropical ecosystems. Practical convenience may also introduce bias in the study of particular plants and animals easily grown and bred under laboratory conditions. Intellectual preference must also play a part.

Certain features of fungi make the study of their ecology both fascinating and difficult. The potential importance of fungi in ecosystems, particularly as symbionts (mycorrhizal fungi) and as decomposers (saprophytic fungi) has been discussed by Harley (1971) in his fascinating survey "Fungi in Ecosystems". He pointed out that "to ask general questions about the magnitude of their intervention in nutrient cycles and energy flow, is premature". Quite a lot of ecologically important work has been done since these words were written, however we are still far from appreciating the extent to which these plastic, versatile and invasive organisms contribute to the "running" of the ecosystems of which they are a largely hidden part. This is not because their contribution is likely to be particularly insignificant or their roles obscure. Rather, that those features which particularly characterise their mode of life makes them extremely difficult to study qualitatively, let alone quantitatively. Ingold summarizes some

of these difficulties. "Modern ecology demands a quantitative approach..... with fungi the vegetative part, the mycelium, is so intimately associated with its organic substratum that estimation of the amount present is almost impossible. Further, although most fungi can be cultivated and their physiological potential assessed, it is extremely difficult to obtain meaningful estimates of their activity in nature."

Not only are fungal hyphae of a few microns in diameter and "intimately associated" with a natural substratum difficult to see, but unless they are sporulating they are impossible to identify. Unless macroscopic fruit bodies are produced microscopic examination of the original substrate is necessary; the nature of the substrate may make this rather difficult. The fungus may not be sporulating at all. This means that the material must be brought out of the field into the laboratory and cultured until recognizable reproductive features are produced. This leads to a sort of 'fungal uncertainty principle' where the fungus can either be seen in situ and not identified or identified in culture and its relationship with the original substrate obscured. These difficulties are explored in more depth in Section 4.1.

There is still a certain amount of bias in laboratory techniques left-over from the large amount of work done in plant pathology. Gradually techniques which attempt to mirror 'ecologically appropriate' conditions in the field are being used, but there is still a temptation to use 'conventional' fungal methods, and to derive inappropriate ecological conclusions from such work. The comparison of methods in such interesting ecological studies as Suberkropp and Klug: "Fungi and bacteria associated with leaves during processing in a woodland stream" (1976) and Barlocher and Kendrick: "Dynamics of the fungal population on leaves in a stream" (1974). illuminate these difficulties and contribute to ecologically sound methodology (see sections 3 and 4).

1.2. What is an aquatic fungus?

Ingold gives a useful survey of aquatic fungi and their origins in his 1974 Hooker lecture. He points out that less than 2% of all fungi are aquatic. There are two main groups. Primitive species, 'originally' aquatic and with motile zoospores, form the first group. These are the 'water molds', also commonly known as aquatic phycomycetes, with species in the Chytridiomycetes and oomycetes. The other group contains members of the Ascomycotina and Deuteromycotina (including aquatic Hyphomycetes) and just a few members of the Basidiomycotina. These are re-migrants; they have re-evolved to an aquatic existence from terrestrial forms.

Ingold also points out that "It is difficult to characterize an aquatic fungus, and indeed to define the aquatic environment itself" (in Gareth-Jones 1976). However a working definition of both is useful and important when discussing and speculating on the ecological role of various fungi.

Some habitats are obviously aquatic: the sea, estuaries, rivers, streams, lakes and ponds. There are also 'micro-aquatic' habitats, found within the terrestrial environment; for example the thin water films around leaves in a litter layer, and the aqueous phase between soil particles. Fungi are found in all of these.

Definitions of aquatic fungi are far more complicated. Usually an aquatic fungus is regarded as one which survives, grows, sporulates and is dispersed "completely submerged in a large volume of free water such as streams and rivers, ponds and lakes" (Ingold 1976). This definition excludes other fungi associated with aquatic environments. For instance, certain fungi which grow below the water surface but sporulate above it- the so-called aero-aquatic Hyphomycetes survive and grow on submerged,

decaying leaves but need to be exposed to the air for the production of their distinctive, usually coiled conidia; these are dispersed on the water surface. Also certain lower fungi, parasitic on higher plants, rely on thin water films for dispersal of their motile zoospores (eg. Potato blight - Phytophthera infestans).

In aquatic Hyphomycetes the complete life-history can and does occur completely submerged in a large volume of free water - usually a fast-flowing river or stream. However, some species can also be found in micro-aquatic habitats (see especially 2.3) and some survive (under laboratory conditions) in a dry environment.

In this account, whenever aquatic is used with reference to either a fungus or an environment the above definitions and exceptions should be kept in mind.

1.3. What are Aquatic Hyphomycetes?

1.3.1. Hyphomycetes: a collection of imperfect fungi.

The characteristics of sexual reproduction are very important in the taxonomic classification of most fungi into divisions, classes, families, genera and species; also in their subsequent identification. Thus in the division Ascomycotina sexual reproduction involves the production of a special sac-like cell known as an 'ascus', characteristically containing eight 'ascospores'. This feature distinguishes the Ascomycotina from the division Basidiomycotina for instance, where four basidospores are borne externally on a special structure known as a basidium. Classification within the Ascomycetes is based on differences between and similarities amongst the structure of the spore containing asci and the fruiting bodies which produce, support and protect them.

The class of fungi known as the Hyphomycetes belong to the division Deuteromycotina, also known as the Fungi Imperfecti. Sexual reproduction is unknown and the division provides a convenient place for those fungi which do not have a recognised perfect, or sexual state. Most reproduce asexually, some produce only sterile hyphae. The ability to reproduce sexually may have been lost during evolution, (and replaced by other methods of recombining genetic material). The 'imperfect' fungus may have a 'perfect' state, a member of the Ascomycotina, Basidiomycotina or Zygomycotina (see 2.1.6). The two states may require very different combinations of environmental conditions for survival and the connection between them not detected. Even if such a connection is discovered the two "different" fungi may be so well known in their own right that the original classification is retained. Thus, of the thirty or forty Penicillium species in the class Hyphomycetes, the majority do not reproduce sexually; a few do and are also known as Eurotium

species. These are typical members of the Ascomycotina.

For convenience, therefore, a variety of fungi of varying origins, known and unknown, are arranged in groups known as 'form-genera'. These correspond roughly to 'true' genera in the rest of fungal classification. The member species of form-genera are related by certain characteristics of their asexual reproduction; spore shape, colour and septation and the way in which the spores are produced. Because of convergent and divergent evolution in the adaptation to certain modes of existence, membership of the same form-genus does not imply any phylogenic relationship. In fact, very closely related perfect fungi may have quite dissimilar modes of asexual reproduction, whilst two very similar species of imperfect fungi may have rather different perfect origins.

Many members of the Hyphomycetes are ubiquitous and successful fungi. They are microscopic, reproducing rapidly and profusely with no elaboration of fruit bodies, the spores (conidia) being produced externally on more or less specialised hyphae (conidiophores). Adaptation to a particular environment can be traced in the evolution of spore types for efficient dispersal; for instance, the small, round, pigmented, aerially dispersed spores of the terrestrial Penicillium species; and the relatively large, colourless, branched spores of many aquatic species.

1.3.2 Aquatic Hyphomycetes: Ingold's first study

'Aquatic Hyphomycetes' could be defined as those fungi in the class Hyphomycetes found habitually growing and reproducing in water. However, since 1942, when Ingold published his paper "Aquatic Hyphomycetes of decaying alder leaves", the term has taken on a rather more specific meaning. It embraces a number of freshwater Hyphomycetes, of various genera, most of

which have a rather characteristic spore morphology, and ecology. Although truly aquatic in that they can complete their life cycle submerged in water, some are also found on land and in temporary waters where their versatility helps them to survive (see 2.1.4 and 2.3.6.1).

Many of the features common to all or most aquatic Hyphomycetes can be found in this first account of Ingold's.

He describes several unusual and distinctive spore types which he found suspended in the waters of a small English stream. All the spores were colourless (hyaline), thin walled, and relatively large for fungal spores. Most were branched, the majority of these having four radiating arms forming a roughly tetraradiate organisation. Long 'filiform' spores, with a curvature in more than one plane, were also found. Only one of the spores had a more conventional spherical spore shape (see Figure 1.2).

The form and size of these various spores was remarkably constant and this suggested that each belonged to a separate fungal species, or at least to a separate spore stage of a fungus. As Ingold comments in a later review (1975b) "shortly after I encountered this remarkable assemblage of fungi it became clear that the mycoflora to which they belonged had not previously been recognised, although a few species had been noted and rather imperfectly described (de Wildeman 1893, 1894, 1895). However it was necessary to allocate most of them to new genera or species". Soon Ingold discovered the source of these spores. Microscopic examination of the surface of decaying, submerged alder leaves revealed 'forests' of conidiophores bearing the easily recognisable spores and identifying them as Hyphomycetes. Such sporulating fungi could be found on leaves taken straight from the stream in summer and early autumn. Even when the conidiosphores could not be seen, submerging such leaves in shallow water

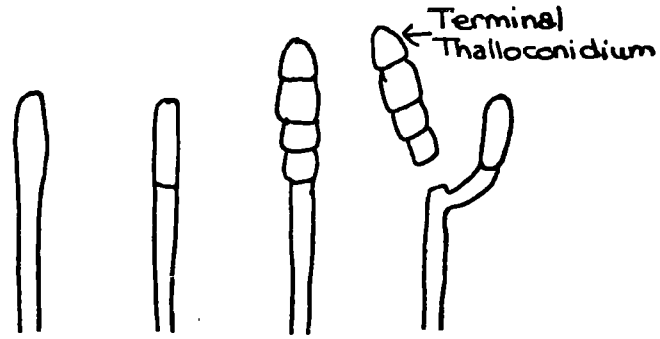

Fig 1·1

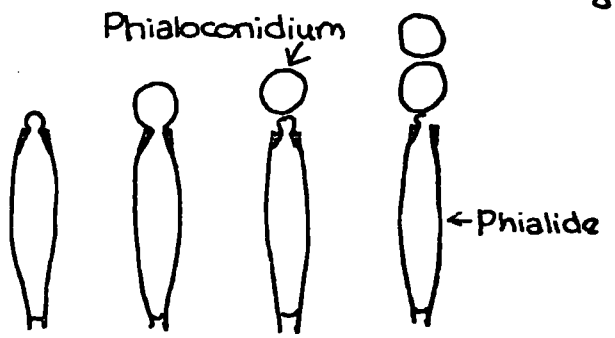

Diagram illustrating fundemental differences between terminal thalloconidiam and phialoconidia (after Ingold)

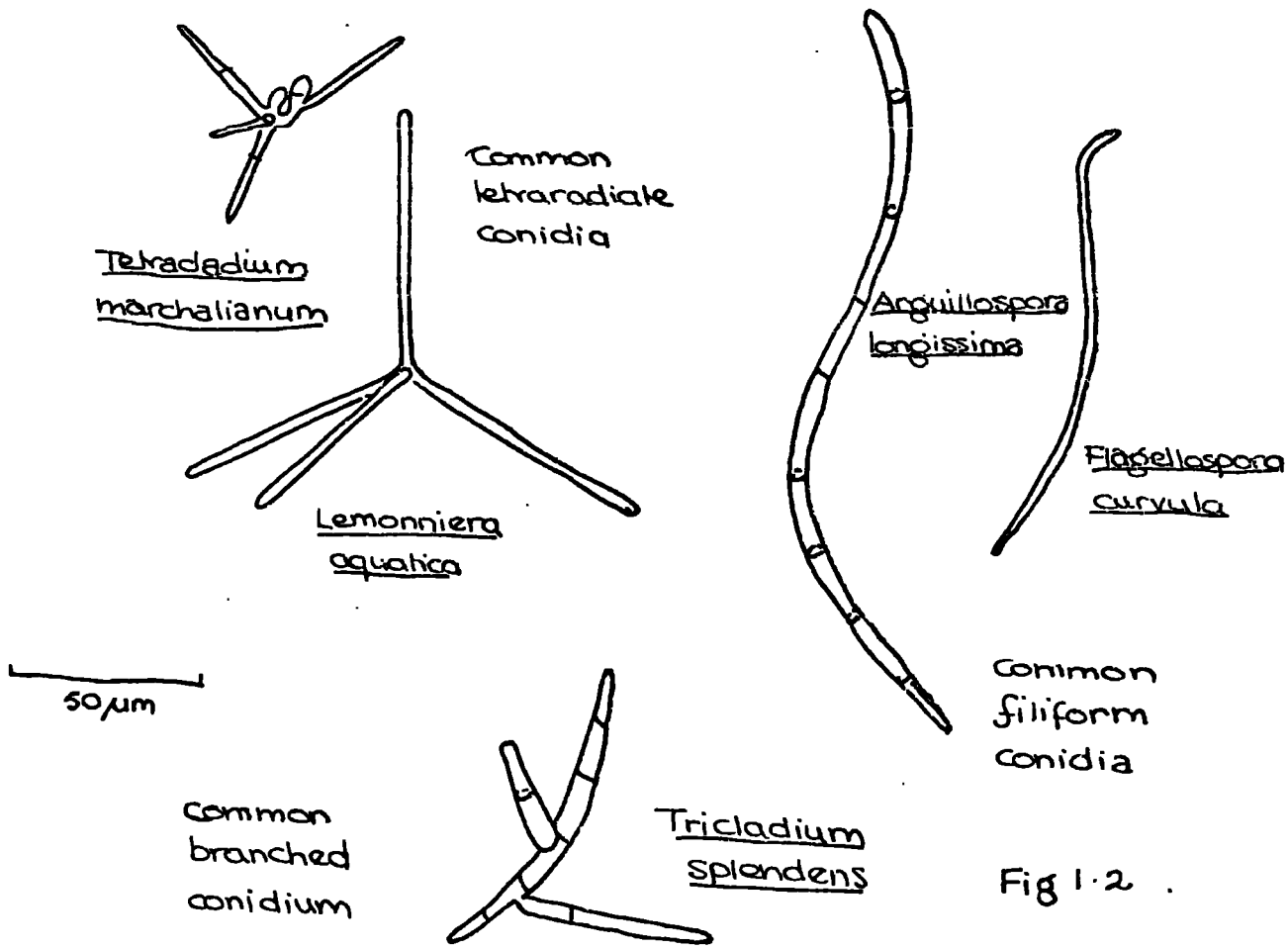

Fig 1·2

in the laboratory stimulated the hidden, vegetative hyphae to produce rich crops of conidiophores and attached spores after a day or so. Leaves that had reached the skeleton stage of decay produced particularly abundant crops of spores.

He found that the branched, septate, vegetative hyphae tended to grow within the veins and petioles whilst the conidiophores projected from the surface of these structures.

These fungi dominated the fungal flora of the alder leaves all year round. Very rarely did a leaf fail to produce the characteristic conidia and conidiophores. Usually several species (an average of 5) were found growing on the same leaf.

In the laboratory, most of the species found grew on the solid medium provided (malt agar) producing characteristic dense colonies of various colours (see 2.1.5). Ingold found that all but two would not sporulate in this medium and strips of the compact colonies had to be submerged in water before spores were produced. This seemed to confirm their aquatic nature.

In his discussion Ingold advances several ideas which he and other workers have worked on and elaborated since. Two are particularly important and interesting: "In considering the Hyphomycetes of the submerged decaying leaves the remarkable shape of the spores in most species forces itself on the observer's attention..... in most genera it consists of four branches diverging from a common point... when, however, the development of the branched spore in the different genera is considered the mode of formation is so different that one is driven to the conclusion that the similarity in general spore shape is the result of parallel evolution" (see 2.1.4). Also..."it would seem likely that (the spore shape) has survival value.... since such a spore would probably stand a much greater

chance of becoming entangled in the decaying leaves of the stream bed". (see below).

1.3.3 Characteristics of aquatic Hyphomycetes: typical and exceptional

Research since 1942 has confirmed "that there is a vast flora of aquatic Hyphomycetes growing on submerged decaying leaves and twigs of broad leaved trees and shrubs in well-aerated water" (Ingold 1976). Many new genera and species have been described, with many new variations on the basic tetraradiate, branched, and filiform spore shapes. The number of different substrates, habitats and geographical locations from which they have been reported has increased greatly. The role of aquatic Hyphomycetes as saprophytes peculiarly well adapted to an existence in stream ecosystems has emerged; with interesting studies from both the lab. and the field on their potential and actual ability as decomposers under a variety of circumstances.

In his "Guide to Aquatic Hyphomycetes" (1975a), Ingold gives a brief survey of the biology and ecology of these fungi in a succint 2000 word introduction.

Spore shape has always been one of the most obvious and interesting attributes of aquatic Hyphomycetes. Several papers and reviews investigate and emphasize the development, significance and possible origins of the predominant spore shapes. The constancy in spore form and size within a species means that many can be fairly confidently identified from these features alone.

Certainly spore shape is an important criterion in classification, though the setting up of form-genera also depends on the way in which these spores have been produced form their conidiophores. In the Hyphomycetes

there are several ways in which hyphae are elaborated to produce spores (see 2.1.2). Most aquatic Hyphomycetes exhibit one of two of these methods. These are simply illustrated in Figure 1.1 and succintly described by Ingold (1975b): "In the main there are two types of conidia in aquatic Hyphomycetes: terminal thalloconidia (formerly referred to as aleuriospores) and phialoconidia. They are quite distinct.....

In the thalloconidium the primordium is early delimited from the conidiophore by a cross-wall. Thereafter it undergoes further development until fully formed. Then the conidiophore either does not produce another conidium, or, if it does so, this is formed at a different level or to one side of the first.

The phialoconidium is formed from the apex of a special cell or phialide that is usually fatter in the middle than at the two ends. No separating cross-walls are formed until the conidium is fully mature. Thereafter a new one is produced by the phialide at exactly the same level as was the first".

It is interesting to note that several 'pairs' of genera occur in the aquatic Hyphomycetes; in both members the spores follow a very similar pattern of development geometrically, but in one genus this is as a phialoconidium, and in the other a thalloconidium. (see Figures 2.1 - 2.9). It is very unlikely that the members of such a pair were related in the past. It is more likely that they reached their similar end products independently by adaptation from less elaborate phialconidial and thalloconidial forms. These "ancestors" may have been terrestrial Hyphomycete fungi and have counterparts in present day species. Nilsson, in his comprehensive review of aquatic Hyphomycetes speculates on several possible evolutionary pathways from terrestrial species of the appropriate developmental type, through aero- or semi-aquatic types to truly aquatic forms (for a fuller explanation see

Section 2.1.4 and Nilsson 1964).

Much has been written on the convergent evolution that seems to have produced these elegant spore shapes, showing variations on the two main themes. The phenomenon is especially interesting since it is not restricted to the conidial shapes found in aquatic Hyphomycetes. Aquatic imperfect fungi of the Sphaeropsidales, aquatic species of Basidiomycete (freshwater and marine), an unusual species of insect parasite (an Entomopthora species), an aquatic yeast, and certain algae all produce branched or tetraradiate propagules; whilst some aquatic fungi belonging to the Ascomycotina produce long, filiform ascospores (see 2.4.1).

Ingold had already suggested that the prevalence of such distinctive shapes indicated some biological significance for them; some way in which they contributed to the successful adaptation of the fungi to their optimum environment, the swift flowing stream.

Two theories were put forward to explain the value of conidial shape to dispersal: "The first suggests that conidia of this kind are likely to remain longer in suspension in water than oval or spherical ones of the same mass and thus achieve a wider dispersal" (Ingold 1976). Webster (1959) investigated this theory by experiment. He found, however, that neither branched nor threadlike spores differed appreciably in sedimentation rate from spores lacking processes. In fact, in a fast flowing stream or river, the setting out of small particles such as spores is very slow compared with the forward movement of the water. Thus spore shape does not appear to contribute to transport down stream. "The second theory is that the tetraradiate conidium is related to impaction on an underwater object: in other words that it may be regarded as a microscopic anchor". (Ingold 1976). Webster also studied this theory experimentally. This he did by setting up a 'trap' (a vertical glass rod) and allowing a known concentration of conidia

(at a known rate of flow) to sweep past it. Microscopical examination allowed the conidia deposited to be counted. Trapping efficiency (at higher rates of flow) was found to be greater in the tetraradiate conidia than for the elongated forms, whilst these values were much higher than for rounded, more conventionally shaped spores. Thus branched and long threadlike spores appear to have effectively solved the problem of 'initial anchorage' being readily trapped by potentially suitable substrates as they sweep by. This feature is obviously of great ecological significance. a stream existence. Bärlocher and Kendrick (1974) are of the opinion that this particular adaptation is one of the most important "selective advantages" that the aquatic Hyphomycetes possess and that it has been largely responsible for the world-wide success of these fungi in flowing waters.

Another striking feature of aquatic Hyphomycetes is the rich, dense accumulation of their spores found in the persistent cakes of foam and scum trapped upstream of barriers such as twigs. Such foam occurs particularly after heavy rain and is formed by turbulent water in waterfalls and rapids. "It seems that air bubbles, as they move through the water, trap the conidia and these are retained in the foam. They are slightly denser than water but, once incorporated in foam and scum, are held firmly by surface-tension forces" (Ingold 1976).

Foam and scum therefore provide an extremely useful tool to the student of aquatic Hyphomycetes. Muira (1974) surveying the stream spora of Japan retrieved 84 spore types by examination of scum, far more than could be produced by examination of the substrate. Nilsson (1964) found that 75% of all swedish species of aquatic Hyphomycete could be retrieved from a single foam sample taken from a large stream. The sporas of streams can

be rapidly surveyed without the further mycological treatment needed when examining most substrates for fungi. However, Iqbal and Webster (1973a) have shown that tetraradiate species are picked up in foam more efficiently than others, so the quantitative relationship between species in foam will not be accurate. (for other cautions on the use of stream spora see section 2.3.6.2)

Since Ingold's paper in 1942 the aquatic Hyphomycetes have emerged as a successful and widespread group of fungi: "Aquatic Hyphomycetes form a highly distinctive, abundant and ubiquitous flora on submerged decaying leaves of trees and shrubs of many kinds in well-aerated streams." This is the habitat and substrate with which these fungi are particularly associated. Their abundance in this typical environment can perhaps be indicated by the following comment on spore load in an English river: "Conidia in the river often reached a concentration of over 10^3 litre and occasionally the value rose to over 10^4. These are very high figures when compared with what aeromycologists have found in the air where values as high as 10^2/litre are rare." Whilst Nilsson (1964) noted that in streams particularly favourable to the growth of aquatic Hyphomycetes, every single submerged tree leaf showed evidence of colonization.

Most of our knowledge of the aquatic Hyphomycetes has been gained by studying these fungi in their typical stream environment. However, they are found elsewhere - on different substrates and in different habitats - though not necessarily in such variety or profusion.

For instance, aquatic Hyphomycetes are found in various aquatic habitats such as lakes, ponds and estuaries; in changing habitats such as temporary pools and flooded areas; and in terrestrial habitats such as the forest leaf litter layer (section 2.3.3). The latter is particularly

interesting. Many of the species found are rather 'versatile', and may produce a different spore shape on land than they do submerged. However, many typically aquatic species have also been found on land, where their specialized spore shapes have no obvious use. This topic is treated in more detail in section 2.3.6.1.

Aquatic Hyphomycetes are not only found on leaves though physiological studies (2.2.3) and field observations(3.3) have confirmed their importance on this substrate, in fact: "there is a strong likelihood that aquatic Hyphomycetes are the main agents bringing about decay in the submerged leaves of deciduous trees and shrubs which contribute such a large organic input to rivers and streams in autumn". (Ingold 1975b). They are also common on wood, and may also be found on such natural substrates as grass leaves and conifer needles. On grass leaves a very few species are found, but in profusion; whilst on conifer needles several species have been found, but not in abundance.

Aquatic Hyphomycetes have also been found growing on such artificial substrates as string, cloth and glass. This illustrates their ability to use dissolved nutrients in the surrounding water (see 2.3.2).

These fungi are particularly well studied in temperate areas. This is partly due to the fact that most interested scientific workers live in these areas; partly due to the enormous influence that the autumn-shed leaf input, colonized and decomposed by aquatic Hyphomycetes, has on the ecological functioning of temperate streams. These streams rely on the import of leaves to supply energy needs that cannot be satisfied by primary production. The aquatic Hyphomycetes aid the exploitation of the energy and the nutrients that these leaves contain. Aquatic Hyphomycetes are found in streams and rivers all over the world, where they perform essentially the

same function. However, the massive seasonal input of leaves into temperate streams and the way in which aquatic Hyphomycetes have adapted to active growth and sporulation in low winter temperatures make them a particularly interesting and important habitat to study. (see 3.2).

Not only are aquatic Hyphomycetes important in their own right as decomposers of leaf material but much recent attention has been focused on their interaction with those invertebrates which, by devouring autumn-shed leaves and their attached fungi,are also very important in the exploitation of this imported material. "Hyphomycetes as intermediaries of energy flow in streams" (Barlocherand Kendrick 1976) is a concise review of recent work on this fascinating ecological topic. They comment: "The digestion of higher plant remains by animals is in most cases mediated by micro-organisms. This is often an absolute necessity since most invertebrates lack cellulase and lignases"; cellulose and lignin make up a large part of plant material. Bacteria and fungi, including aquatic Hyphomycetes, by colonizing and decomposing leaves and converting them into their own biomass make them a more attractive and valuable food source, aiding the exploitation of the energy and the recycling of the nutrients they contain (see section 3.4).

To conclude: laboratory experiments and field study have revealed that the ecological group of fungi known as aquatic Hyphomycetes are particularly well adapted to an aquatic existence in running waters, being found in streams amd rivers throughout the world. They are particularly important as colonizers and decomposers of leaves in temperate streams, where they contribute to the efficient functioning of the stream ecosystem.

2. AQUATIC HYPHOMYCETES

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2.1. The Taxonomy and Morphology of the Aquatic Hyphomycetes

2.1.1. Introduction

In discussing the taxonomy and morphology of the aquatic Hyphomycetes as a group, three accounts are particularly useful:

Nilsson, in his 1964 review "Freshwater Hyphomycetes - taxonomy, morphology and ecology", provides an extensive exploration of the basis of hyphomycete taxonomy and puts much emphasis on the relationships between taxonomy, parallel evolution and morphology. He discusses at length the different ways in which the predominant conidial shapes are produced and speculates on their relationship to and development from fungi whose spores are less adapted to an aquatic mode of life.

Ingold's fascinating Hooker lecture on "Convergent evolution in aquatic fungi: the tetraradiate spore" (1975b)gives lucid accounts of spore development and adaptation. Ingold's book "Guide tothe Aquatic Hyphomycetes" (1975a)is "essentially an atlas or picture-book to assist in the identification of the aquatic and water borne microfungi". It gives clear illustrations of spores and spore development and allows a rapid survey of morphology.

In the last decade or so much has been discovered about the role of aquatic Hyphomycetes as an ecological group, and of their importance in the first stages of leaf decay in running waters. The number of species found has increased enormously and much detailed work has been done on spore development in an attempt to refine the relationships between similar species. Attempts have been made to link these imperfect fungi with their perfect forms; some of these attempts have been successful (section 2.1.6.). These rapid developments in the study of aquatic Hyphomycetes are coupled with a continuing controversy over the basis of classification on the Hyphomycetes

as a whole, and over the nomenclature associated with various types of spore development and spores produced (see Section 2.1.2.).

This means that classification in the aquatic Hyphomycetes is rather dynamic with genera being renamed, or new ones set up, with species moving from one genus into another. Intensive work on certain genera has refined their characteristics and set their classification and identification on a firmer footing, with keys being set up (see for example Iqbal (1972) on the genus Anguillospora, and Descals et al (1977) on the genus Lemmoniera).

A note on nomenclature

The following terms have and will be used in this account:

spore: a general term for a reproductive body; used in algae, ferns, etc. as well as in fungi.

conidium: a spore produced by a fungus belonging to the Deuteromycotina or Fungi imperfecti; an asexual spore.

sometimes the shorter term spore has been used where conidium would be strictly more accurate (Kendrick 1971).

sporophore: conidiophore these terms are synonymous and refer to the "spore bearing structure in Hyphomycetes - a more or less differentiated hypha" (Nilsson).

sporogenesis: the development of a spore on its sporophore; the way in which it develops.

phialide: a special cell often fatter in the middle than at top or bottom ("flask-shaped"): it forms part or all of a conidiophore and from its tip conidia known as phialoconidia are formed.

phialoconidium: (phialospore) a conidium produced by a phalide. The distinction between this, a terminal thalloconidium and other conidia named after their mode of production, is useful in the classification of the Hyphomycetes.

terminal thalloconidium: (formerly "aleurospore") 'thallospore' has been used as a general term for a spore that is part of the hypha bearing it: a terminal thalloconidium is a conidium produced terminally from the hypha that bears it (conidiophore) without any special structure being involved (contrast phialoconidium)

phialoconidia and terminal thalloconidia are the most common types found in the aquatic Hyphomycetes (see Section 1.3 and Section 2.1.2.)

2.1.2. Classification in the Hyphomycetes

The problems associated with classification and taxonomy in the Hyphomycetes in general have been outlined in section 1.2. Nilsson (N64) gives a history of classification in the Hyphomycetes; the various attempts that have been made to impose some sort of order on the many and diverse forms of conidia and conidiogenesis encountered.

"In classifying the Hyphomycetes many systems have been published, used and discussed. It must always be emphasized that a system for this group of fungi will necessarily be artificial until we know the 'perfect' stage of the species - if there is such a stage in all of them". (N64)*. Saccardo (1880) divided the class into four main divisions based on very general external features of the spores produced. Subdivisions were based on shape and septation of spores. Thus the whole system was founded on varying and unstable characters which should be regarded as secondary differentiating characters" (N64). In fact, study of many species of imperfect fungi has shown that spore characteristics such as these can change in response to changes in their environmental conditions. In certain aquatic Hyphomycetes, for instance, a different spore shape is produced when the fungus sporulates on agar, from that produced when it sporulates completely submerged. The typical "aquaspores" produced in this way, however, are remarkably constant in shape.

In the 1940's the primary division of Hyphomycetes was into dry and slimy

*I shall only refer to one work of Nilsson's - his extensive 1964 review. I shall therefore use this convenient abbreviation.

spores, with aquatic spores conveniently - if rather inappropriately - included in the latter. Hughes (1953) used more stable characters in his classification, separating characteristics being based on the form of the sporophore and sporogenesis with spore types being named after their manner of development.

Nilsson adopted a very similar system, by-passing an alternative classification produced by Subramian (1962) which attempted to use more conventional taxonomic criteria. Nilsson felt this was less appropriate since the Hyphomycetes are strictly form -species based on form-genera. His system is a rearrangement, with various refinements, of its predecessors and includes numerous references to their terminology in an attempt to clear up synonyms and ill-defined terms. Thus it contains eight main groups, named after developmental spore types (in the manner of Hughes). He points out that the majority of aquatic Hyphomycetes belong to two of these groups: they either have phialospores and belong to the Phialosporae or have aleurospores (now known as terminal thalloconidia) and belong to the Aleurosporae.

The most recent, concise account of Hyphomycete classification and taxonomy is in Ainsworth, Sparrow and Sussman (The Fungi, Volume IVA: A Taxonomic Review 1968). Here the authors use "four independent character sets" in the arrangement of form-genera:

1. Saccardoan spore group
2. General arrangement of conidia
3. Colour of conidia
4. Type of conidiogenous cell

"Conidial states are grouped into form genera for convenience in identification and nomenclature. The species included in a form genus are related to each other by the form of their conidia and conidiogenous apparatus but not necessarily by phylogeny". Here therefore we have a useful terminology rather than a "true" taxonomy.

Kendrick (1971) attempts the formidable task of overhauling Hyphomycete taxonomy. This is obviously an extremely useful book but is beyond the scope of this discussion. Some of its recommendations have already been implemented: others are slowly being absorbed.

Thus improvements and refinements have been made, and continue to be so. More sensible arrangements - in the light of possible phylogeny - have been attempted: more accurate nomenclature has been adopted. Much controversy still exists however, and there is a legacy of synonymous terms to plague the unwary student of imperfect fungi.

2.1.3. Classification in the Aquatic Hyphomycetes

Ingold was the first person to recognize and describe these fungi as a group (1942). A few of the species he found had previously been formally described and named; some of the other spore shapes had repeatedly been mistaken for algae. He continued to make a major contribution to new species discovered - in an astonishing range of geographical localities - and was responsible for setting up many of the aquatic Hyphomycete genera: seven new genera were described in his first study alone. Some of these have gone through the inevitable refinements and shifting around of species common in fungi classified in this way.

To generalize: many of these genera are exclusively aquatic; neither do terrestrial genera contribute many species that are truly aquatic (as far as is known). It is likely however, that close relationships exist between genera in these two main ecological 'divisions'. There are several versatile 'borderline' species which can thrive both aquatic and terrestrial environments. Increasing ecological and taxonomic knowledge of aquatic Hyphomycetes in particular, and Hyphomycetes in general, will no doubt clarify these relationships.

A look through Ingold's clearly illustrated and set-out guide (1975a) is a good way of seeing how the "four independent character sets" have been used in erecting the various genera. The main 'split' is between genera based on thalloconidia and those based on phialoconidia. Ingold regards this as a fundamental division; a few species produce spores by both methods. Other differentiating features are based on the geometry of conidial development: in some species the arms of branched spores develop simultaneously, in others they develop one after another; in tetradiate forms the spore may be attached by an arm, or near the point where the arms diverge. Fortunately "development is easy to study since it can readily be followed in hanging drop cultures. Further the developmental patterns tends in each species to be very precise, the conidium achieving its mature form with geometrical precision" (Ingold 1975b). Examples of Ingold's sequential developmental drawings are given in Figure 2.10.

2.1.4. Topics relating to spore morphology: an integrated account.

Because classification in aquatic Hyphomycetes is so closely associated with spore form and spore development - and because of the ecological significance of spore shape - it is very difficult to separate discussion

of taxonomy and classification from spore morphology and speculation on the origins of these aquatically adapted fungi. An integrated account is therefore proposed using the useful framework provided by Nilsson. He divides his discussion into five sections each of which deals with a particular group or range of spore shapes. This account follows the same sequence with an additional subsection (Vd): much of the substance of the account is derived from Nilsson, augmented where necessary from more recent work. Most species commonly found in foam and on plant material in this country are discussed; other species have been included to make the account of diverse shape and development more complete. For a full list of genera and species Ingold (1975a) and several other more recent papers would have to be consulted.

All discussions on aquatic Hyphomycete spore morphology, including this dissertation, contain rather concentrated information. It is difficult to appreciate verbal accounts and descriptions without looking at illustrations of the spores in question. Each spore group discussed here has a companion figure with illustrations of spores from that group. These are mature spores; the difference between thalloconidia and phialoconidia can be appreciated as can the different ways in which the spores are attached. Geometry of development for all the spores mentioned has not been included; excellent illustrations of timed stages in development in various species can be found in Ingold's original 1942 paper, his 1975 guide and the 1975 lecture on tetraradiate spores. An example is given in Figure 2.10.

Nilsson includes a comprehensive series of illustrations to accompany his text. One set compares similarly shaped spores (some hypothetical) of various origins; he also includes a series of illustrations of mature spores in the various genera known at that time.

Group I: Species with spores having a more or less spherical to oblong shape

Spherical spores are typical both in aerially dispersed spores ("dry spores") and in many species bearing "slime spores": these are dispersed diversely, for instance by rain or animals, sometimes by streams and other waters. In aquatic Hyphomycetes there are very few species which have this type of spore. Two reasonably well known but not particularly abundant species (from different genera) are Dimorphospora foliicola and Margaritospora aquatica.

D. foliicola produces two very different types of spore, from the developmental point of view. Its "dominant" spore is a thalloconidium. When produced in water these spores are ovoid to reniform: these latter curved spores float on the surface of the water before sinking, the others remaining submerged. D. foliicola can also produce tiny round phialoconidia; perhaps these can act as aerially dispersed spores (though Ingold (1975a) notes that so far germination of these has not been successful). Thus this fungus is adapted to aquatic and aeroaquatic habitats and may even be dispersed aerially.

Margaritospora aquatica has a spherical phialoconidium which, when produced submerged, has more or less pronounced outgrowths of a tetraradiate organisation. However, spores produced aerially on agar, or on a sporophore which projects through the surface of shallow water, may become elongated, curved, and septate: these spores can float on the water surface. (figure 2.1).

Dactylella is not a purely aquatic genus. It includes terrestrial nemotode-catching fungi. Its aquatic species are a motley collection. The thallocondia of the most common aquatic species, D. aquatica, has a rounded, 3-dimensional, diamond-shaped spore: however Nilsson has noted in this fungus a tendency to develop marked processes, similar to those in the

aquatic spore of M. aquatica (Figure 2.1.).

Group II. Species with spores having a cylindrical or bacilliform to elongated shape.

This spore shape is common in many terrestrial parasites and saprophytes on attached and fallen leaves. Distribution of these species may be aided by rain water, dew etc. There are no typically aquatic species with this spore type and it does not seem suitable for submerged distribution. Species of the terrestrial genus Cylindrocladium often appear on leaves from very shallow or almost dried out water, enormous numbers of floating spores being produced.

The common aquatic Hyphomycete Heliscus lugdunensis (see Group V, Figure 2.5) produces an aerial phialoconidium as well as the well known clove-shaped aquatic spore. The aerial form is cylindrical to slightly clavate and can also be produced in shallow water where it floats readily (Figure 2.2). On agar glistening spore masses are formed. "The resemblence of the aerial spore of H. lugdunensis to those of a species of Fusarium * already been pointed out by several authors". Spore heaps and the production of phialoconidia are characteristic of both Heliscus lugdunensis and Fusarium and contribute to this resemblence. It is interesting to note that the perfect state of H. lugdunensis is an ascomycete, a species of Nectria. Some of the other species in this genus have "imperfect" stages which produce Fusarium spores. Nilsson also suggests that an adaptation to aquatic habitat in Fusarium may also have led to the development of more typically aquatic spore-shapes: "A typical tetraradiate member of Fusarium "origin"

* a ubiquitous terrestrial genus of saprophytic imperfect fungi having straight to curved, septate spore.

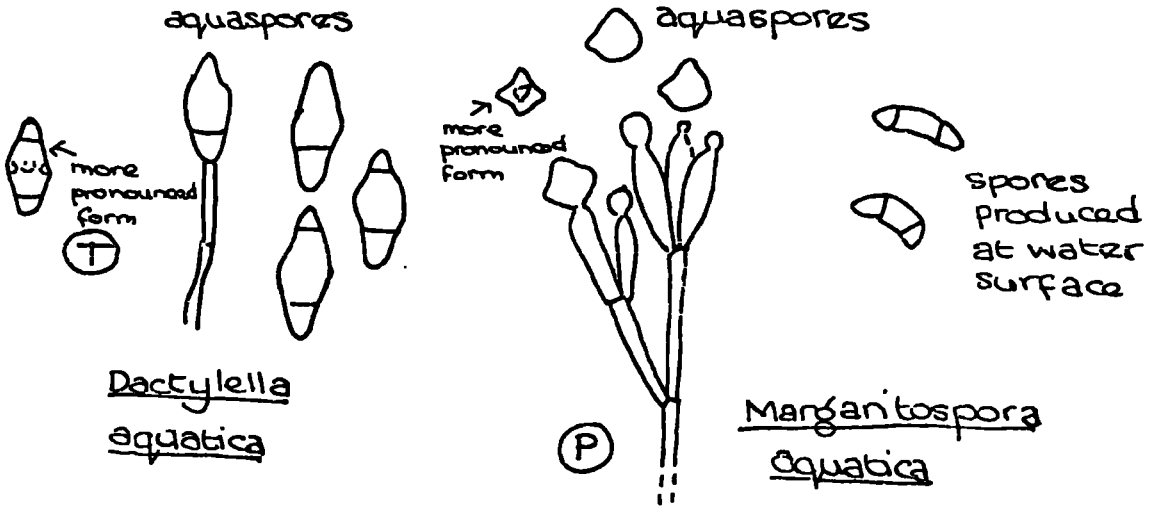


Fig. 2.1 Group I spores

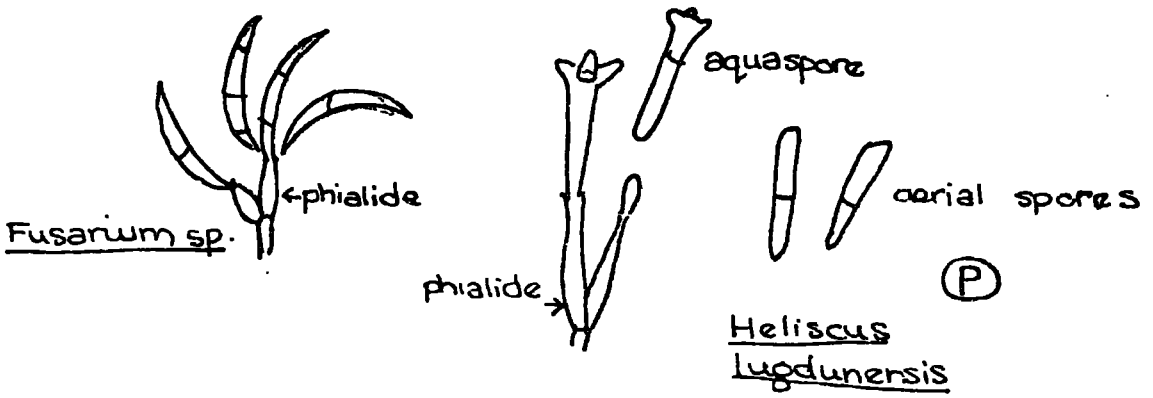
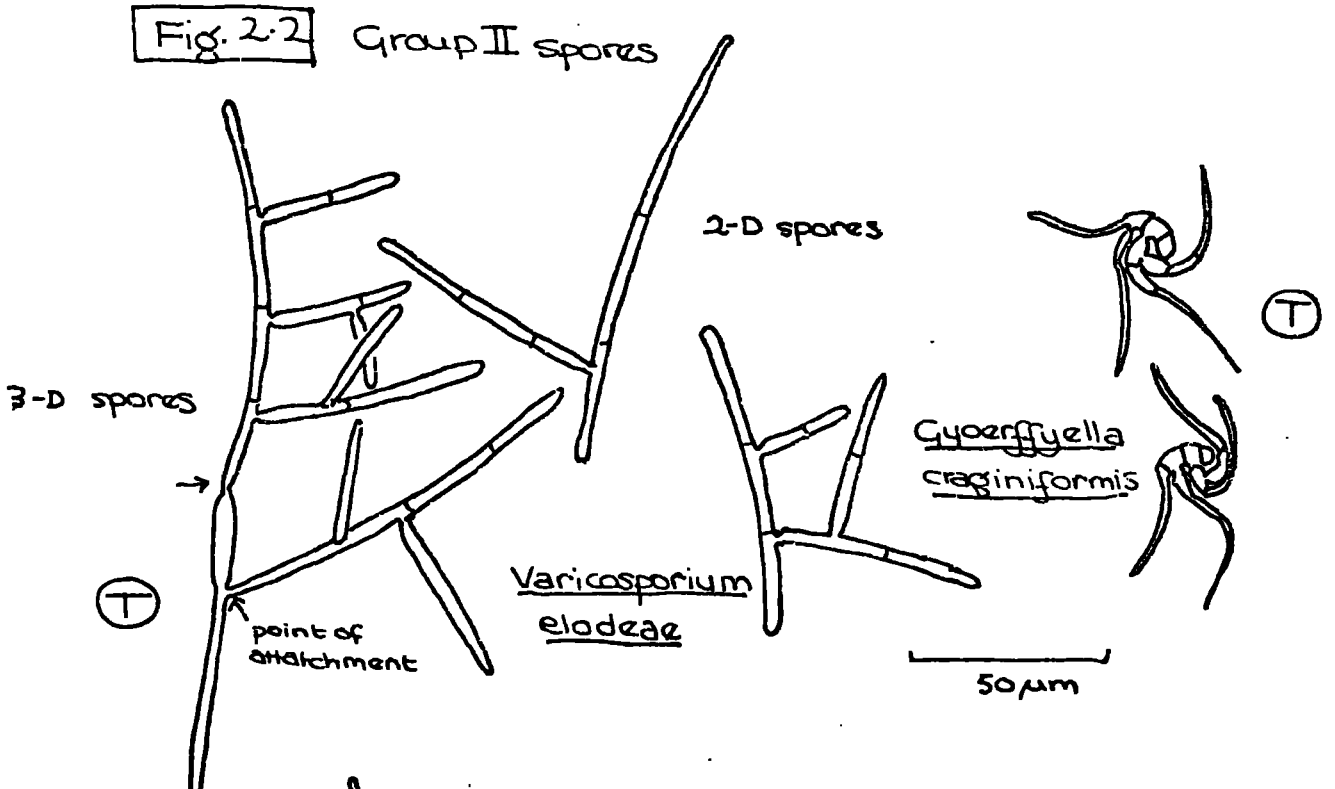


Fig. 2.2 Group II spores



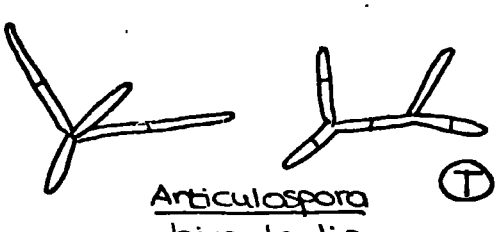


Fig. 2.3 Group III spores

would then possibly be searched for in eg. the phialosporic genus Clavatospora"(See Figures 2.2 and 2.5).

Group III: Species with spores consisting of a branched or budding system, usually in one plane.

Nilsson points out that very few Hyphomycete species have spores consisting of "a complex of numerous branches". However several known species of aquatic Hyphomycetes do belong to genera based on this type of spore; there are also several "unknown" species found only as spores. Some of these have " 3-dimensional structure" and are discussed in Groups Vc and Vd. A few species have spores which branch in one plane only: they are 2-dimensional.. Nilsson gives speculative illustrations of the possible development of both these types from simple hyphal budding systems.

The thalloconidia of Varicosporium elodeae have frequently been found in foam samples in Britain; they have been likened to Chinese characters. This species exhibits an astonishing range of spore shape due to variations in the number of arms and the amount of branching. It has been found totally submerged, in soil, and in moist terrestrial habitats. When the spores are produced totally submerged they may branch in more than one plane. Nilsson, who found this fungus typically in stagnant pools, emphasises its aero-aquatic features: the two dimensional spore developed in such circumstances floats readily on the surface of water (Figure 2.3).

The thalloconidium of the well-known aquatic Hyphomycete Articulospora tetracladia has two forms (Figures 2.3 and 2.6): a typical tetraradiate organisation A.tetracladia f. tetracladia, and A. tetracladia f. angulatum in which the arms are in one plane. Nilsson associates this latter spore type with stagnant water and has found it produced on plain agar.

Aberrant spores of the simple branched species Tricladium angulatum produced under aerial conditions have a two-dimensional rather than the normal three-dimensional form.

Particularly striking arrangements of branching in one plane are exhibited by the genus Gyoerffyella. G. craginiformis has a thalloconidium resembling a shrimp with curved, tapering branches (Figure 2.3).

It is interesting to note here that the clamped, branched spore Ingoldiella hamata, assumed to be the asexual spore of a basidiomycete and found both in scum and on decaying leaves, has two forms. A tetraradiate arrangement of its tapering, septate arms is produced below water whereas a Tricladium - like spore, branched in only one plane, is produced at the surface of the water.

Thus the species described above in Groups I, II and III, although found in truly aquatic habitats are also associated with semi- or aero-aquatic conditions, and even terrestrial habitats; some species however, when submerged, produce spores which are more obviously adapted to an aquatic existence.

The next groups include species found typically, if not invariably, in submerged conditions; their spores exhibit structural features shown to be of adaptive advantage in an aquatic environment (see section 1.2). The re-iteration of such shapes in different genera and in other aquatic fungi is stressed.

Group IV: spores with elongate, more or less bent to sigmoid spores with the curvature usually in more than one plane.

Slightly bent and sigmoid spores are found in terrestrial imperfect fungi - in fact they are quite common - but they are usually bent in one plane; they are two-dimensional. Similar spores in the aquatic Hyphomycetes when typically developed submerged in water, show a curvature in more than one plane.

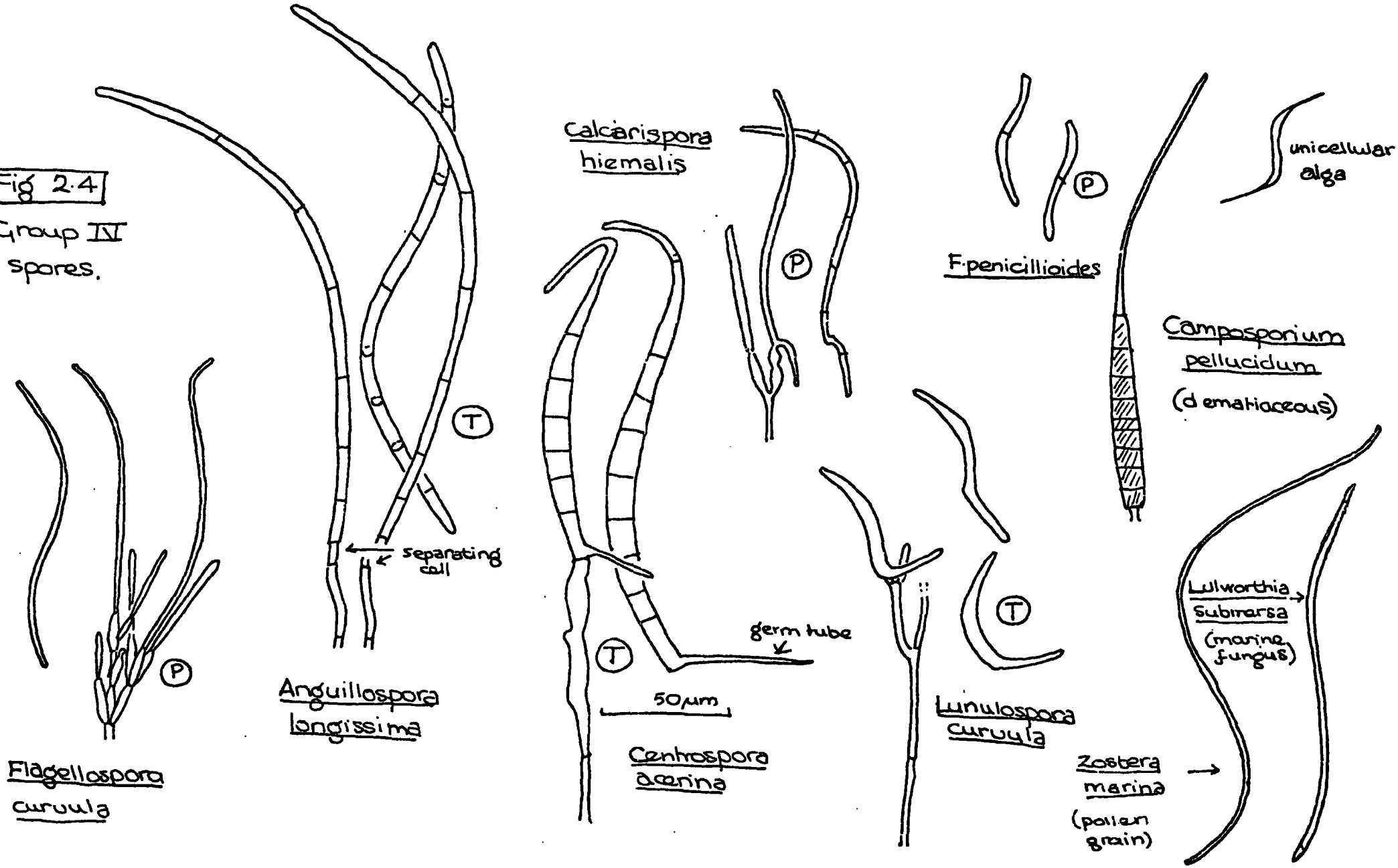
Flagellospora curvula is an extremely common aquatic Hyphomycete with a world-wide distribution. Its long, thin sigmoid phialospores exhibit this feature very clearly: the shorter sigmoid spores of F. penicilloides are less frequently found in running water. F. curvula also has a Nectria perfect stage and thus its genus could be related both to Heliscus and Fusarium. Cultures of both these Flagellospora species on solid agar produce spores bent in one plane only (Figure 2.4).

The thalloconidial equivalent genus to Flagellospora is that of Anguillospora. This genus contains many closely similar spores and has recently been augmented and revised by Iqbal (1972). This is the dominant aquatic genus with a true sigmoid shape. Species of Anguillospora occur in almost any type of water, but usually in running water (Figure 2.4).

An interesting spore shape is exhibited in the pair of genera Centrospora (thalloconidial) and Calcarispora (phialoconidial) (Figure 2.4). Here a curved or sigmoid spore is augmented by a basal process - described by Ingold (1975) as the first germ tube - which develops whilst the conidium is still attached. The genus Centrospora is a mainly terrestrial one; the species most regularly associated with the aquatic environment, C. acerina can also live as an unspecialised parasite of carrots, etc. - even aquatic isolates have been found to be pathogenic (Iqbal and Webster 1969).

Fig 2.4

Group IV spores.



The thalloconidia of the genus Lunulospora, crescent shaped or sigmoid, and curved in more than one plane, show resemblances in shape to Anguillospora, but in attachment to Centrosporina. "Relationships between Calcarispora, Centrospora and Lunulospora may be theorized" (N64).

F. curvula, Lunulospora curvula and Anguillospora species are all very common - the two former species have a world-wide distribution and their spores are found abundantly in foam. This fits in well with the hypothesis that this shape is a suitable adaptation to the aquatic environment. Although Webster (1959) found that the "trapping efficiency" of three-dimensional curved spores was less than that of tetraradiate and similarly shaped spores, it was still far superior to that of the other spore shapes tested. Further evidence of parallel evolution in spore morphology is provided by the more or less sigmoid, elongated spores produced by several aquatic ascomycetes. Several species of algae also exhibit this shape, whilst the most interesting instance outside the fungi is the very long, thin, s-shaped pollen grain of the marine grass Zostera marina (Figure 2.4).

Group V: Spores with a more or less well developed tetraradiate organisation

This is the most common spore form in the aquatic Hyphomycetes; the three-dimensional curved spore being the next most abundant.

Ingold has written much on the subject of convergent evolution towards a common, well adapted spore shape. He has illustrated his discussions with many examples from the aquatic Hyphomycetes, though the appearance of similar shapes in other aquatic organisms supports his case. His most recent exposition on this theme is the highly readable and comprehensive "Convergent evolution in aquatic fungi: the tetraradiate spore" 1975b.

He states that "the developmental pattern of the 4-armed conidium is so dissimilar in the different genera [of aquatic Hyphomycetes] that the conclusion seems inevitable that this type of spore is the result of convergent evolution".

There are three 'sets' of fundamental differences in the expression of the tetraradiate form, with genera based on these differences. The first is the development of closely similar phialoconidial and thalloconidial forms. The second involves the attachment of the conidium to its conidiophore; most species have spores attached either by the tip of one arm, or near the point of divergence of the arms. It is interesting to note that in the non-hyphomycete fungi that have developed this type of spore shape, both types of attachment occur. The third involves the geometry of development of spores- the order in which the different parts appear as the mature spore develops from its primordium.

In discussing the variations in the final form and development of the tetraradiate spore both Ingold (1975) and Nilsson emphasize the existence of those phialoconidial and thalloconidial pairs of genera with closely similar form and geometry of development. A particular pair of species, one from each genus, often show a striking resemblence to each other. Nilsson also introduces the idea of a 'top species'. In each group of fungi developing a more or less tetraradiate organisation in its particular way, there is a species in which this shape has been refined to its most simple and elegant; this is also true in the species with a 3-dimensional sigmoid spore shape. Such species are probably better adapted to an aquatic existence, they "are in fact dominant and also the most widespread of the freshwater Hyphomycetes". (N64)

Group VA: Spores attached by the tip of one of the four arms

Group VAi: In many of these spores the first formed part or arm dominates. It is usually wide, often clavate (see Figure 2.5).

Phialoconidia are produced in the genera Heliscus and Clavatospora: H. lugdunensis has already been described. Although Clavatospora has a more 'advanced' tetraradiate organization - in C. longibra chiata, for instance - the more versatile H. lugdunensis appears to be more common.

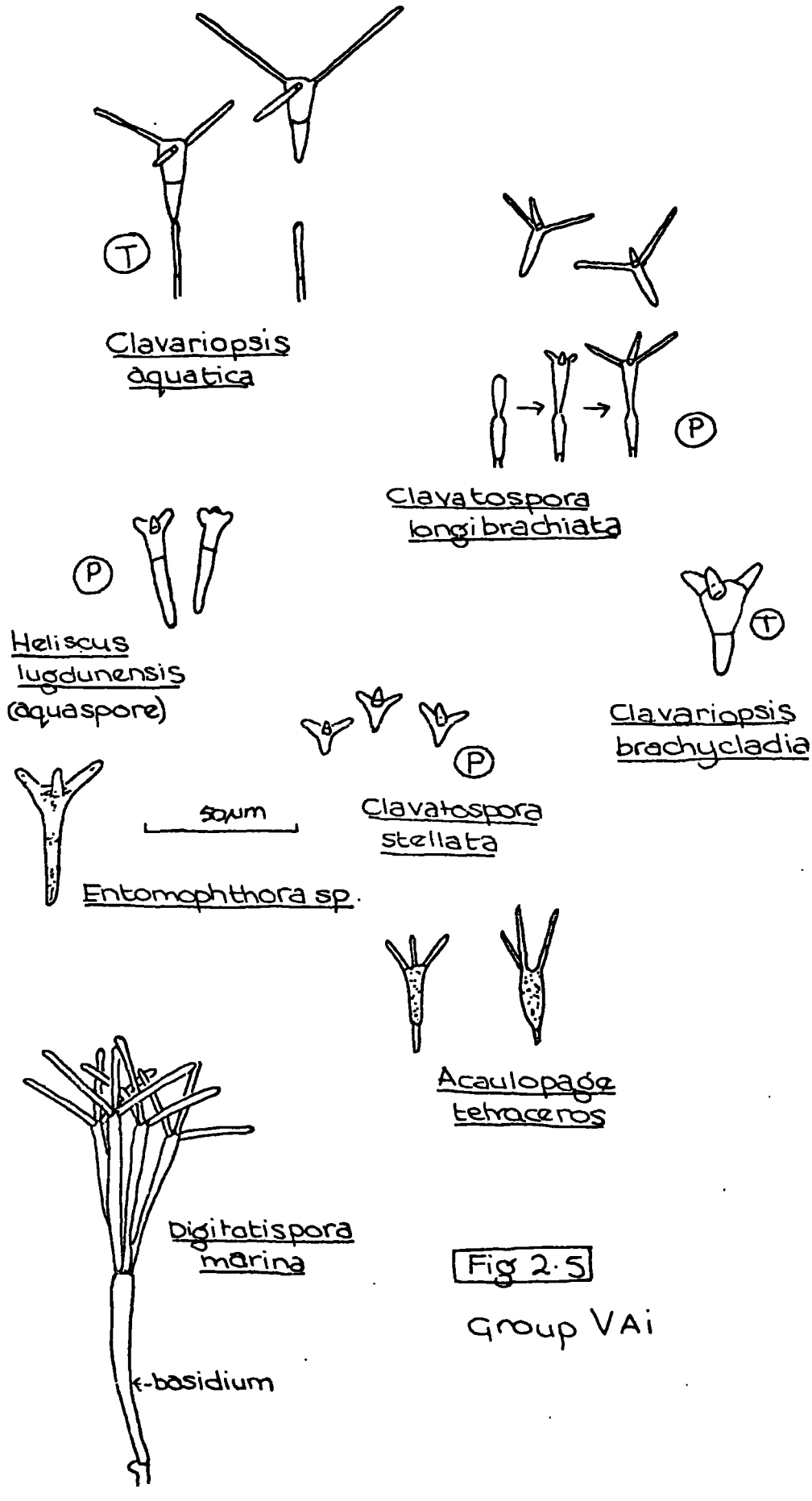
The thalloconidial equivalent species to C. longibra chiata is Clavariopsis aquatica: this is the 'top' species, the most common and widespread fungus with this type of development. The same pattern is echoed in other groups of fungi: Digitatospora marina (a marine basidiomycete), Torpedospora marina (a marine ascomycete) and Acaulopage tetraceros (an amoebae - catching aquatic fungus) all have spores strongly reminiscent of those described above.

Group VAii: in some spores "the first part forms together with the second a more or less continuous bent main axis. On the continuous axis, or at the point of insertion of the second arm two other divergent arms arise at the same level". (N64).

This precise, rather long verbal description is embodied in the small, delicate 'wide awake' phialoconidia of Alatospora acuminata, and in its large, elegant thalloconidial equivalent Tetrachaetum elegans (Figure 2.6). A. acuminata - the only phialoconidial species in this group - is very common; its spore is abundant in foam and its distribution worldwide. The large spored T. elegans, however, is rather rare.

There are several other genera with thalloconidia in this group: Articulospora and Tetracladium both have well-known species.

A. tetracladia, apparently adapted to both an aquatic and a semi-aquatic existence, is very common and widely distributed. Spores with 2 to 5 arms,


T
Clavariopsis aquatica
P
Clavatospora longibrachiata
P
Heliscus lugdunensis (aquaspore)
T
Clavariopsis brachycladia
P
Clavatospora stellata
50µm
Entomophthora sp.
Acaulopage tetraceros
Digitatispora marina
basidium
Fig 2.5
Group VAi

arranged in various ways, are produced. But a simple tetraradiate arrangement is commonly observed, with the form described in Group III as the main variation (Figure 2.6). The submerged spores of Ingoldiella hamata (Figure 2.6) are reminiscent of the tetraradiate spores of A. tetracladia.

Rather unusual spores are produced in the genus Tetracladium. As in A. tetracladia a first arm is developed and then several buds develop in strict order. Some of these produce long "arms", others remain blunt processes. In T. marchalianum these long arms form a well defined tetraradiate arrangement and the processes are distinctive but reduced to knobs (Figure 2.6). This species is far more abundant than other members of the genus whose tetraradiate shape is less developed, in fact Nilsson states that together with F. curvula it is the most common and widespread of aquatic Hyphomycetes.

The fungi in this sub-group are distinctive in their clear exposition of the tetraradiate shape. Many of them are extremely common: Nilsson recalls that in Webster's impaction experiments (1959) A. acuminata, A. tetracladia and T. elegans "together with Tricladium splendens were the most effectively trapped spores (mean of all observations) closely followed by other common tetraradiate types such as Lemmoniera aquatica [Group VB] and Clavariopsis aquatica [Group VAi]".

Several algal genera produce structures of similar shape to the spores of the fungi included in this group. This helps to explain why some aquatic Hyphomycete spores were once mistaken for algae. Terrestrial genera exhibiting such spore shapes do exist, but are not common.

Group VB: spores attached at or near the point of divergence of the arms

Lemmoniera aquatica is a well-known and common fungus with a clear simple tetraradiate organisation of this type. It is the 'top'

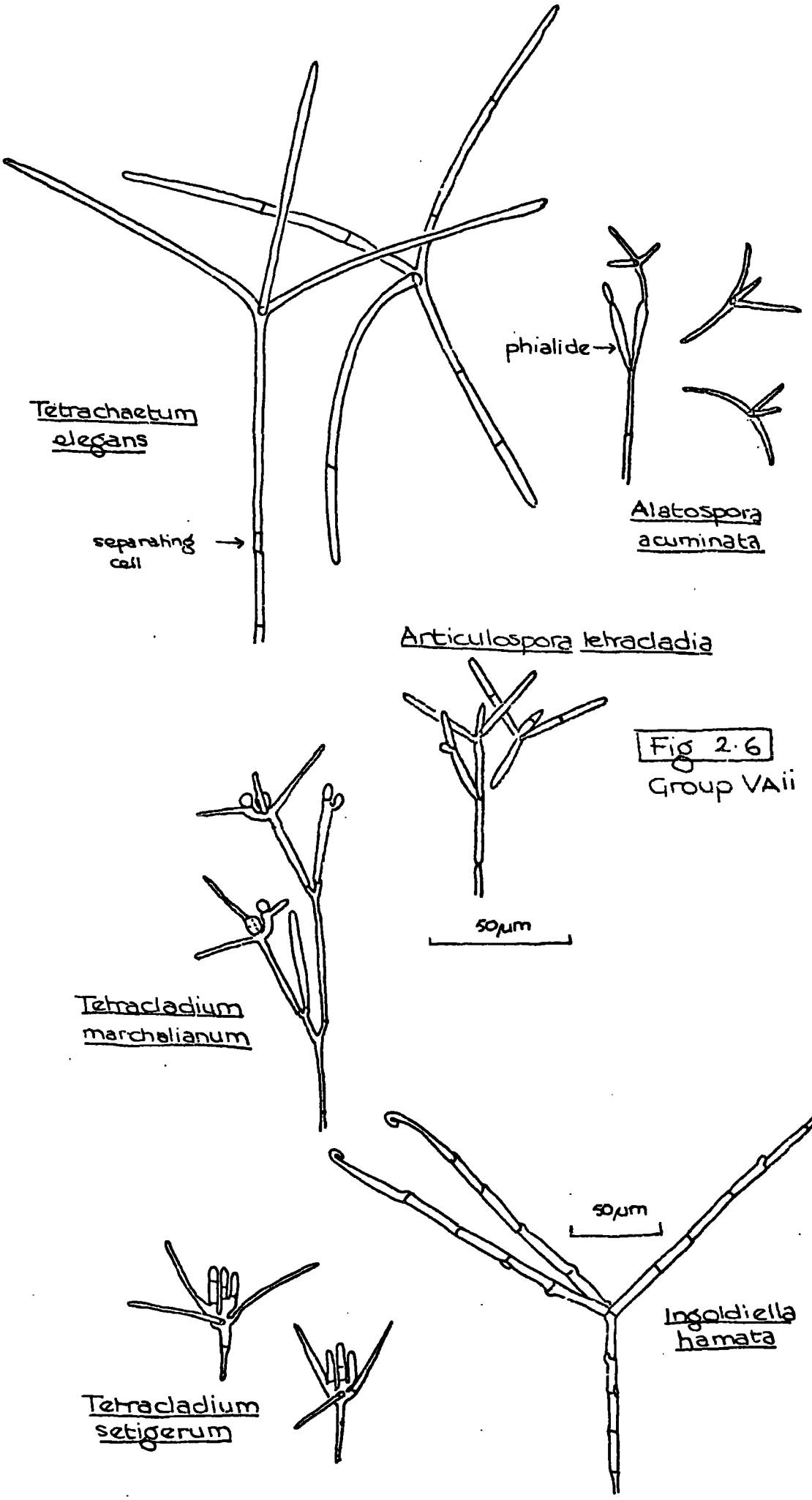


Fig 2.6
Group VAii

phialoconidial species. The phialoconidia of Margaritispora aquatica look remarkably like the primardia of this fungus, (compare Figures 2.1 and 2.7). Tricelophora monosporous, a species found mainly in the tropics, is the thalloconidial equivalent of L. aquatica - it is even more similar to the less common L. filiformis. The enormous spore of Actinospora megalospora forms a giant thalloconidial twin to L. aquatica. There is a remarkable likeness between these huge spores and those of a Basidiomycete, Nia vibrissa, found on driftwood (Figure 2.7). It is interesting to note here the presumed adaptation to aquatic life shown by the spores of certain Entomopthora species.* Tetraradiate, rather dense bodies of variable form, of the same size range as the majority of aquatic Hyphomycetes spores have appeared repeatedly in foam and scum. These were tentatively assumed, in the absence of contrary evidence, to be shelled rhizopods. A recent study by Webster, Saunders and Descals (1978) has dispelled this zoological assumption. Briefly, the more typical crescent-shaped conidia of an Entomopthora species, also found in scum, have been observed to produce predominantly tetraradiate forms when the crescent is submerged in water. The new form of spore is produced almost directly from the 'old' spore. Several similar tetraradiate forms are produced - one of these, attached by the base of an 'arm' resembles the shape and attachment of Group VAi spores such as H. lugdunensis. Another form observed to develop from the arm of the previous type resembles the spores in the present group. Another species of Entomopthora, with fat crescent shaped conidia, develops small projections round the equator of the spore when this is submerged in water. These are reminiscent of the spores of Jaculispora, a tropical aquatic

*these are internal parasites on insects.

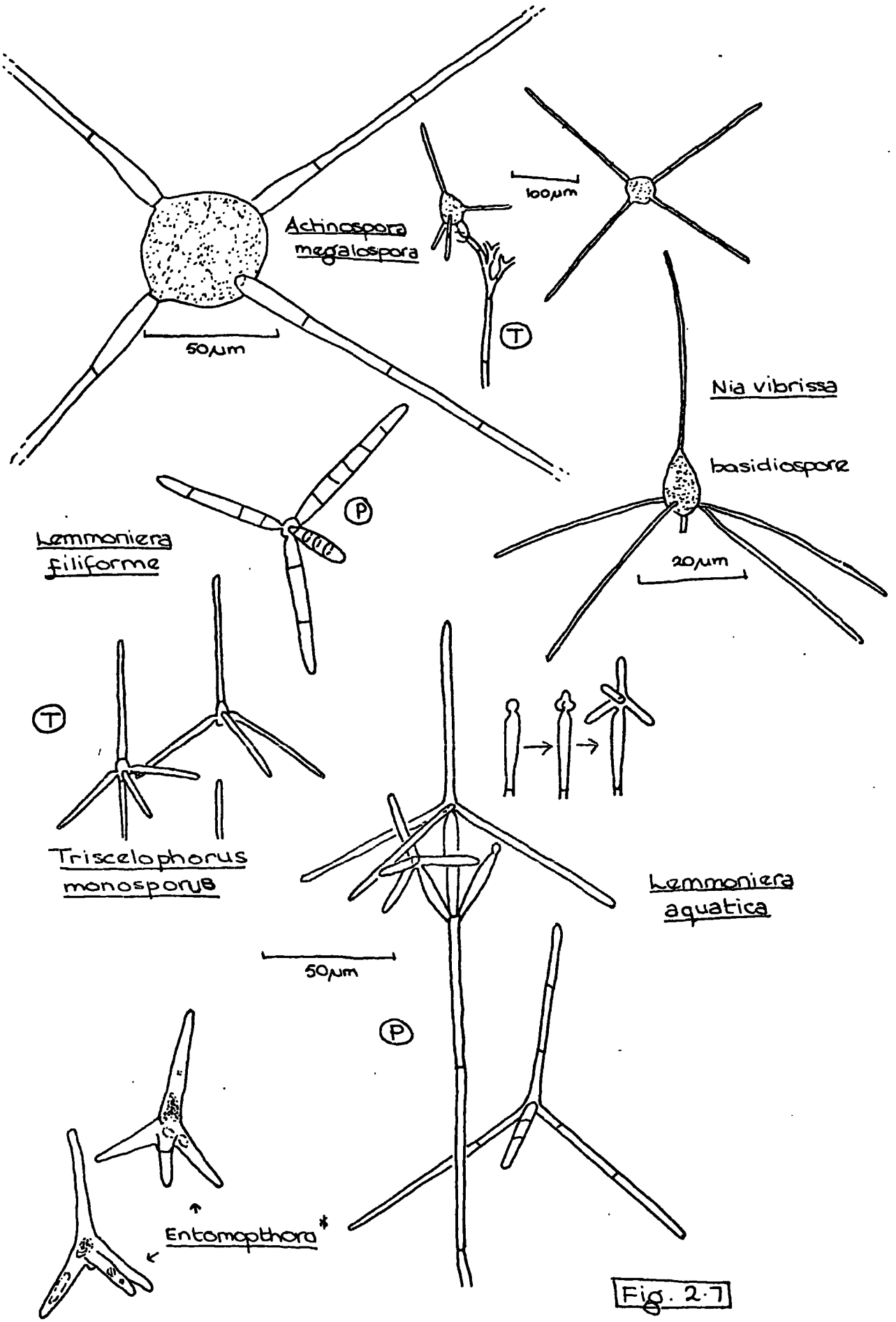


Fig. 2.7

Group VB

* from Webster, Saunders & Descals (1978)

Hyphomycete genus (see Figure 2.7). These are extraordinary examples of parallel evolution.

Several other genera produce various types of branched spore, which can conveniently be described in the next two groups.

Group VC: spores with a main axis and divergent branches but with less typical or without tetra-radiate organisation

This type of organisation occurs in several species; two or more branches or arms arise from a main axis at the same or different levels.

The thalloconidia of the genus Dendrospora are rather spectacular. Several lateral arms, some branched, arise at various levels from the main axis. An extraordinarily similar shaped conidium Dendrosporomyces proliferum has been described by Nawawi et al (1977) who discovered its basidiomycete nature by microscopical study of its cell walls. The genus Tricladium is the most well-known in this group; its species produce rather simple, uncluttered thalloconidia. T. splendens, the most familiar and abundant species, has a curved main axis with two divergent arms projecting from it at different levels (Figure 2.8). It is interesting to note that the spores it produces on plain agar have a shorter, uncurved main axis and are two-dimensional rather than three-dimensional. The efficiency of the curved 'aquaspore' form in initial anchorage has already been pointed out.

Nilsson speculates on the development of simple branched forms such as these from more complicated structures such as the spores of Dendrospora erecta. It is interesting that the branched spores of many genera are produced as terminal thalloconidia, in essence a direct extension of the hyphae that bear them, and presumably reflect their developmental origins.

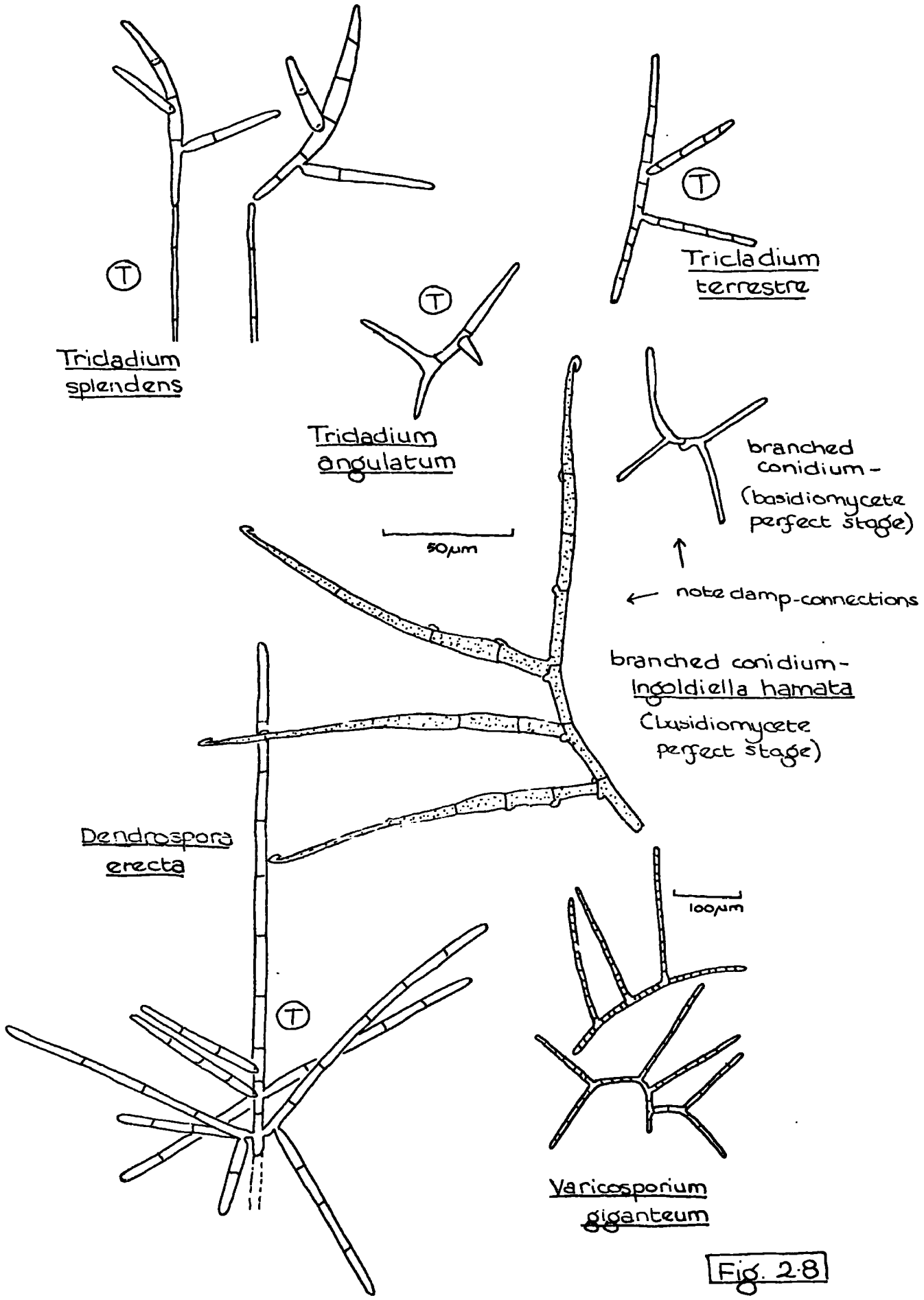


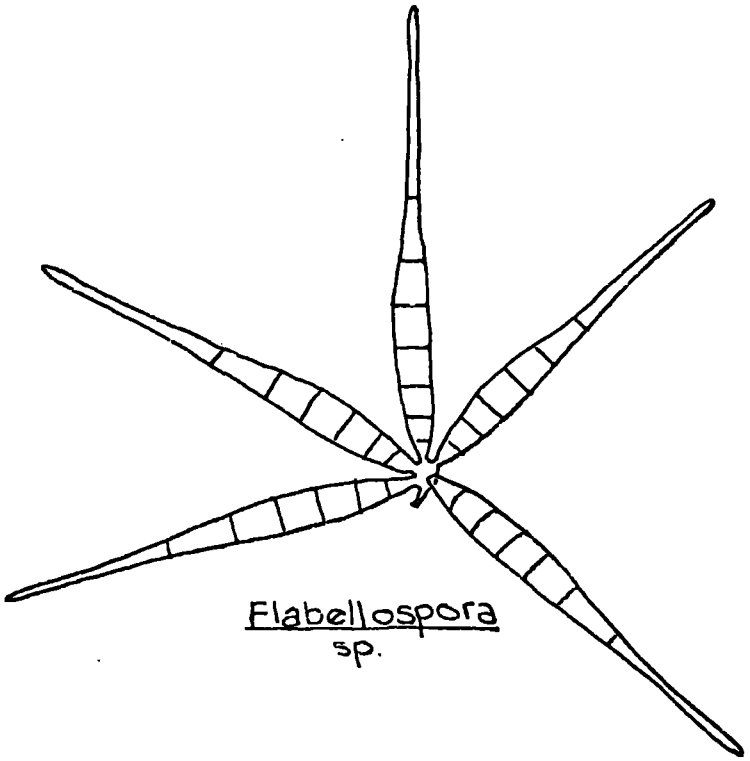
Fig. 2.8
Group Vc

Group VD: A new group has been added here, mainly to accomodate the genus Flabellospora, which has no main axis but a small spherical head from which a number of long straight arms diverge. Thus these thalloconidia differ quite considerably from other types of branded conidia. However, two of these species, F. tetracladia and F. crassa have achieved a tetraradiate organization. Two spores from genera with similar spore forms Isthmotriclaria and Tridentaria, have been included in Figure 2.9 to emphasize their resemblence to F. tetracladia - Ingold points out (1975) that the developmental pattern observed in all three thalloconidia is essentially the same as that of Clavariopsis aquatica, also a thalloconidial species (compare Figures 2.9 and 2.5).

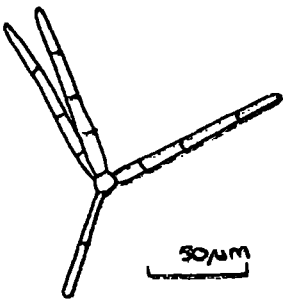
Looking at spore form in the genus Flabellospora it can be speculated that in this genus - as in several others - those species which have achieved a clean, simple branched spore shape have developed from the similar but more cluttered structures, borne either by members of the same genus, or by species in other genera with a closely similar pattern of spore development. 'Intermediate' forms may be extinct or undiscovered. The linking of aquatic Hyphomycete species to their perfect stages may help in tracing such developments (Section 2.1.6). However it is perfectly possible that a progressive adaptation to an aquatic existence, made in the spore-form of a particular species of Hyphomycete with a known perfect stage, could lead to the emergence of a new imperfect species which had left the perfect stage 'behind'.

2.1.5. Vegetative growth, the development of resting structure and germination of spores in aquatic Hyphomyetes

The growth habit and appearance of the vegetative mycelium is very similar in all species of aquatic Hyphomycete. On a natural substratum,



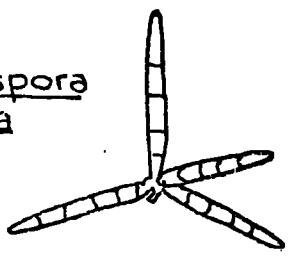
Flabellospora sp.



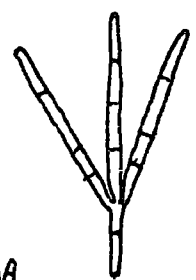
50µm

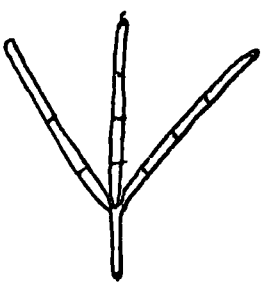
Flabellospora tetracladia

Flabellospora crassa



Isthmotricladia laeensis





50µm

Tridentaria sp.

Fig 2.9

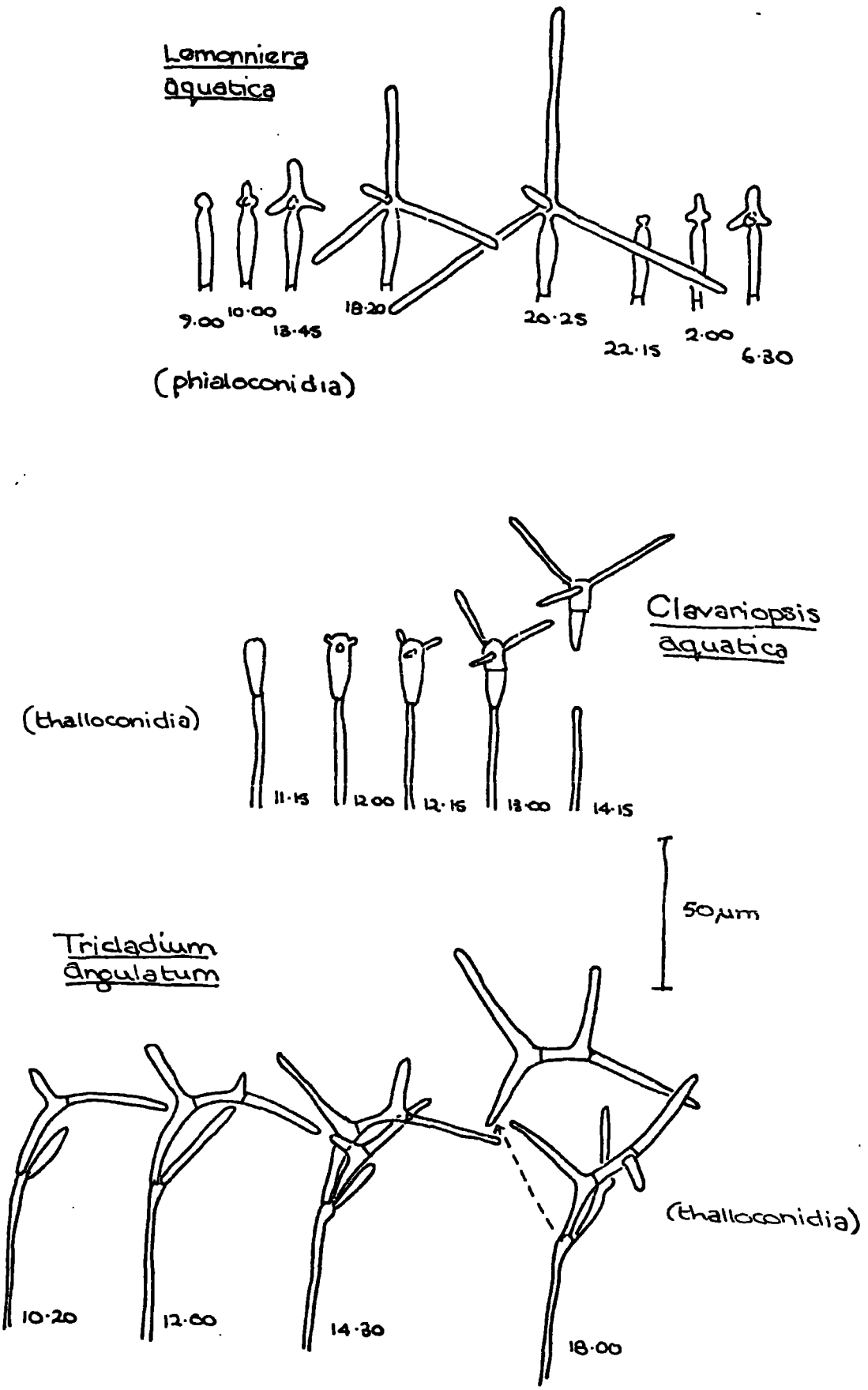


Fig 2.10 Timed camera lucida drawings showing development of three well-known aquatic hyphomycetes from hanging drop preparations (after Ingold)

such as a decaying leaf, superficial and internal mycelia can be found. The superficial growth is often hyaline, whilst close growing surface and internal hyphae may become thicker, and darker in colour. All species have septate hyphae most with a diameter of 2 to 6μ. However giant species such as Actinospora megalospora have much thicker hyphae whilst a few other species, for example Flagellospora curvula, have a finer growth.

Aquatic Hyphomycetes can grow on most conventional laboratory media. Ingold (1942) found that all thirteen species he isolated onto malt agar developed compact colonies with 'mycelial cords' where several hyphae had twisted together. Nilsson commented on the production of strongly curved, short, hyphal branches, especially on plain agar, "which feature usually makes the mycelium recognizable if the aquatic Hyphomycete is grown among other types".

Differences between species can be seen in cultures; characteristic colours may develop as the colonies age. For instance, on malt agar colonies of Lemmoniera aquatica turn dark brown, Margaritospora aquatica colonies stay white, whilst Clavariopsis aquatica forms compact dark olive green colonies with a white fringe.

Many species develop sclerotia in culture; the appearance, size and arrangement of these differs from species to species. M. aquatica for instance, develops small almost black, sclerotia 1-2 mm in diameter scattered all over the colony. "The sclerotium consists of a pseudoparenchymatous mass of nearly spherical cells". (Ingold 1942). Heliscus lugdunensis produces "small dark brown sclerotium-like bodies 0.3 - 1.0 mm in diameter and of irregular shape". Huge masses of spores are eventually produced. Lemmoniera aquatica forms "dark sclerotium-like bodies made up of aggregates of chlamydospores" (Webster 1975). Chlamydospores are usually

thick walled, dark coloured spores which contain food reserves.

They are produced by several species of aquatic Hyphomycete. In L. aquatica these give rise to sporophores and typical spores under favourable conditions. In Clavariopsis aquatica some colonies develop "very numerous little black dots 50-200μ in diameter scattered over the central area of the colony" (Ingold 1942). Some of these are sclerotia, as above; others are pycnidia. These latter structures contain a mass of tiny oval conidia which ooze out of an irregular ostiole. The production of such structures represents a switching of resources to longer lived, tougher structures that may tide the fungus over periods of adverse environmental conditions. They have rarely been observed in nature, however, though Suberkropp and Klug (1976) observed on hickory leaves "thick-walled, dark coloured resting structures.... very similar to those produced in culture by L. aquatica (Ingold 1942)" These sporulated after water incubation.

Spores of aquatic Hyphomycetes suspended in scum, foam and water do not germinate and may remain viable for some time (up to several months). However when the spores touch a solid surface, such as when they settle to the bottom of a specimen tube or Petri dish, they germinate very rapidly. Germ tubes usually develop from the tips of the arms on branched spores, but in many species they can arise from any part: they may arise from each cell in septate spores such as Anguillospora longissima. Nilsson (N64) notes that some species produce appresoria when the germ tubes make contact with the substrate. These, and rapid germination, must aid the initial anchorage of the spore swept against a potential substratum. This stimulation of germination by contact with a potential substrate, and its rapid progression, must be an adaptive feature of these fungi. Nilsson (N64) comments on the self-inhibition of germination demonstrated by large

masses of spores. He found this both in the slimy masses of spores sometimes found on solid media, and on leaves where the sporulation was particularly abundant.

After germination these fungi grow comparatively slowly. This means that, especially on rich media at higher temperatures, they are rapidly overgrown by contaminants: other organisms present in the foam, water or substrate material that is being incubated. This can present great difficulties when recognizing, identifying and isolating these fungi. However at stream temperatures aquatic Hyphomycetes appear to be at an advantage for they have optimum temperatures for growth lying below those of many potential competitors (see section 2.2.2.2).

2.1.6 The Perfect Stages of Aquatic Hyphomycetes.

Perfect stages for Hyphomycetes are usually searched for, and found, amongst Ascomycetes: this is the case for the handful of perfect stages which have been associated with species of aquatic Hyphomycete. There are two main approaches to the search for the related imperfect and perfect stages of the same fungus. One is to attempt to induce the ascigerous stage to develop from pure cultures of aquatic Hyphomycetes. The other is to culture suitable candidates from the Ascomycetes in the hope that characteristic conidia referrable to known aquatic Hyphomycete species will be produced. Suitable candidates may be picked from the same genera as those already assigned, or they may have been retrieved from 'appropriate' moist or submerged habitats.

For instance, Ranzoni (1956) discovered the perithecial fruit bodies of a species of Nectria on a culture of Heliscus lugdunensis. Archer (1971) produced the reverse situation by damp-incubation of a twig bearing Nectria:

pustules of H. lugdunensis eventually developed. Webster (1965) produced the typical conidia of Dactylella aquatica from a submerged colony derived from the ascospores of a wood-inhabiting species of Massarina.

A few perfect stages have been linked directly to an aquatic Hyphomycete imperfect stage of what is, in fact, the same fungus. These pairs have various features in common: with one exception the ascigerous state does not produce ascospores adapted to an aquatic existence; and with one exception those aquatic Hyphomycetes linked to ascigerous stages do not possess spores of tetraradiate shape.

Iqbal (1972) and Ingold (1976) give an account of the discovery of each Ascomycete and Hyphomycete pair. The number of discoveries is increasing and the most up-to-date account will no doubt be found in Webster and Descals 'The Perfect-states of water borne Hyphomycetes'(1978, in press).

Both Iqbal (1972) and Willoughby and Archer (1973) give descriptions of the methods used to induce twigs retrieved from rivers or streams to produce suitable Ascomycetes for further study. Long term damp incubation or gradual drying out of the twigs was required before the fruit bodies appeared.

Interesting taxonomic anomalies can arise from such studies. For instance, very similar species of the ascomycete genus Nectria have been derived from both Flagellospora penicilloides and Heliscus lugdenensis. (Ranzoni 1956, Webster 1959; see Group II section 2.1.4). Almost indistinguishable species of the ascomycete genus Massarina have produced such dissimilar spored species as Anguillospora longissima and Dactylella aquatica (Willoughby and Archer 1973, Webster 1965). Conversely whilst A. longissima has been linked with Massarina, a Pyrenomycete genus A. crassa

has been linked with Mollisia a Discomycete genus. In many ways the perfect stage of a fungus is complimentary to its imperfect stage. Ascigerous stages linked with aquatic Hyphomycetes are usually found or derived from damp or submerged wood. Nilsson believes (N64), and the spores produced suggest, that they are adapted to moist or semi-aquatic habitats. Contrast the aquatic Hyphomycetes which are typically found on leaves and associated with a submerged existence. The development of an ascomycete fruit body and the production of spores may take weeks, maybe months, and be associated with quite an extensive vegetative growth.. The production of conidia in aquatic Hyphomycetes can occur overnight, when leaves are submerged in water in the laboratory - no fruit bodies are involved. Spores can even develop directly from the germ tubes of other spores. The ascomycetes are associated with wood, a 'long-term' and non-seasonal substratum; aquatic Hyphomycetes are typically associated with autumn-shed leaves, a more ephemeral substratum, the bulk of which may disappear before the next leaf fall. The two stages - assuming that under suitable conditions they are capable of producing each other-could, with their different emphasis on substrate and habitat, assist in mutual dispersal over time and space.

Nawawi et al (1977) in a recent paper give an example of a basidiomycete being linked to an aquatic conidial fungus. The branched conidium, rather similar in shape to the spores of Tricladium species and with an obvious clamp connection, has been reported repeatedly from foam (see Figure 2.8). They produced a basidial state - Leptosporomyces galzanii - from the cultures derived from these spores. They also induced the basidiomycete to produce the imperfect stage. They comment that "The fact that the basidial state has been so widely reported on varied substrates under terrestrial conditions whilst the conidial state has been found on leaves in water and in large

numbers in foam raises intriguing problems about the 'amphibian' nature of this fungus". Thus this rather more unusual example nicely illustrates the points made above, as well as giving yet another example of convergent evolution in aquatic imperfect spore shape.

2.2 The Physiology of the Aquatic Hyphomycetes

2.2.1 Introduction

The topics covered in this section (2.2) and in the previous section (2.1), although interesting in their own right, are particularly important as a background to the study of the ecology of aquatic Hyphomycetes: they emphasise those features of the group's morphology and physiology which help in the understanding and study of their ecological role and importance.

The investigations and conclusions dealt with in this section can logically and conveniently be discussed under two main headings.

The Environment: work which assesses survival, growth and sporulation of the fungi when their physical and chemical environment is altered. Experiments on temperature, pH and oxygen supply, for instance may help in understanding why the aquatic Hyphomycetes are typically abundant in some habitats, and not in others, and why; in spite of relatively slow growth, they can overcome competition to become the dominant microbial leaf decomposers in those habitats. The range of conditions they can tolerate may help explain certain less typical occurrences - such as their appearance in terrestrial habitats - and also their absence from or scarcity in other aquatic habitats. It should be remembered, however, that optima and ranges found under controlled, simplified conditions in the laboratory are not unassailable parameters when investigating or predicting the field conditions under which the fungi can survive and thrive. Many physical, chemical and biological factors act together to influence these fungi in the field; it may be unrealistic to expect one specific limiting factor to operate in the field as it does in an artificial laboratory environment.

The substrate: various investigations into nutritional tolerances and preferences have been made. This helps in an understanding of the particular contribution aquatic Hyphomycetes make to leaf decomposition. This is particularly true of experiments which involve growth on various sources of carbohydrate and on their use of dissolved nutrients in the surrounding water. Again the ability to use particular nutrients under laboratory conditions is not an infallible guide to their successful exploitation in the field. Environmental influences and competition with other microbial decomposers may complicate the issue on the natural leaf substratum.

In 1962 Thornton produced a thesis and associated paper (1963) discussing various experiments on the nutrition and physiology of aquatic Hyphomycetes. Comparatively little laboratory work had been done on these fungi previous to Thornton's investigations. He hoped that these experiments would "throw light on the ecology and explain in part the preference for the rather unusual habitat to which these fungi have become adapted".

Nilsson, writing at about the same time (N64), commented on the paucity of work done in this area. He contributes some experiments on the effects of substrate, nutrients, temperature and oxygen concentration with respect to growth and sporulation. Webster and Towfik (1972) and Webster (1975) have investigated the combined effects (physical and chemical) of aeration and oxygen concentration on sporulation. Iqbal's comprehensive thesis "Some observations on aquatic Hyphomycetes" (1972) discusses various aspects of physiology, principally the effect of pH.

These are the principal references discussed in the following account. Many papers on other aspects of the biology of aquatic Hyphomycetes do include observations on their physiology: some of this is practical information on what culture media and conditions can be employed in the laboratory for the isolation and maintenance of pure cultures of these fungi.

It is useful to remember that, even where there is a comprehensive treatment of both nutritional and environmental factors affecting aquatic Hyphomycetes, only a few species have been subjected to detailed scrutiny.

2.2.2 The Environment

The typical habitat of aquatic Hyphomycetes is a well-aerated, fairly fast flowing stream, lined with trees and not particularly eutrophic: "a babbling brook" as Ingold puts it (see Section 2.3).

The success of these fungi in this type of habitat is demonstrated both by spore abundance and by the numbers found growing and sporulating on leaf substrates. This abundance must obviously be linked to the input of leaves to the river or stream. But it is reasonable to suppose that the numbers and the species found depend also on the particular combination of environmental influences that characterize these habitats, and which allow the fungi to make the most of the substrate available.

2.2.2.1 The effects of pH

The effects of pH are rather difficult to separate from other physico-chemical factors, such as the form in which inorganic salts occur in the surrounding water. Most of the conclusions drawn have been from a consideration of both laboratory and field observations.

Suzuki and Nimura (1960, 1961) surveyed a number of lakes in Japan for aquatic Hyphomycetes. They classified the lakes into 3 categories according to the physico-chemical properties of their water, principally pH:

Harmonic lakes: pH between 6.2 and 6.6

Acidotrophic lakes: pH between 3.8 and 5.8

Dystrophic lakes: acid, with humic substances

They found that harmonic and acidotrophic lakes each had their own characteristic range of species and dominant species. Practically no aquatic Hyphomycetes were recorded for dystrophic lakes, except for Varicosporium elodeae. A parallel series of laboratory incubations was carried out using the different types of water. They came to the conclusion that in those waters with sparse records of aquatic Hyphomycetes the formation of conidia had been inhibited. Nilsson (N64) is cautious about the results of this work. "According to culture experiments the authors claimed that some waters should be toxic to some species. The experiments seem however to not be totally reliable".

Iqbal (1972) from his survey of the spora of two river systems, and from consideration of other work, concludes that each river system has its own characteristic spora. This will be due to a variety of physical, chemical and biological influences in combination. However pH probably contributes to this effect. One of the river systems studied by Iqbal, the River Exe and its tributaries, falls into the harmonic category of Suzuki and Nimura; the other, on Dartmoor, falls into the dystrophic category. Very different sporas were obtained: those species most prevalent in each system differed. V. elodeae was one of the few common spores found in the Dartmoor system; it was not found in the Exe system. The range of species found also differed, and whilst the spora of the Exe was rather rich, that from Dartmoor was rather scanty. It must be noted, however, that the number of trees shedding their leaves into the Exe was far in excess of the number contributing to the vegetational input of the Dartmoor system.

Iqbal augments these field observations with a laboratory study on the effects of pH on growth and sporulation in a number of the more common

species. In the field study he deals specifically with the spora of the river system (measured quantitatively; see Section 3.3) rather than the flora; these experiments reveal the relationship between growth and sporulation under certain conditions.

The experiments fall into two groups: the effect of river water on growth and sporulation using species found mainly in one system incubated in water from the other; also the effect of buffered river water, at a range of pH from 5 to 8, on the growth and sporulation of the species found in the river from which the water was collected.

Taking the second group first: an extra investigation was carried out on the effect of different concentrations of the phosphate buffer on the range of pH tolerated by the test fungi. Thorton (1962) in his brief consideration of the effect of pH on two species of aquatic Hyphomycete, had found that the buffer inhibited growth, "detracting from the validity of the results". Iqbal found that increasing the molarity of the buffer could both restrict the range of pH at which mycelial growth occurred, and inhibit sporulation. He also found that the 'optimum' concentration of buffer (that having the least effect) was different for different species. Thus, for Articulospora tetracladia at 0.2 M buffer concentration optimal growth occurred between pH 5.7 and 6.0; with 0.1 M buffer growth was more vigorous; at 0.6 M concentration the range for optimal mycelial growth was extended and sporulation occurred. By way of contrast, Tricladium attenuatum grew equally vigorously in all the buffer concentrations tested, but the pH range for growth was extended by dilution. Fortunately this 'experimental artifact' was recognised, otherwise the results would not have reflected the true response of the fungi.

It is useful here to compare the response of some of the species tested to pH alone:

Articulospora tetracladia:

below pH 6	no sporulation
pH 6.5 - 7.5	optimum for sporulation
pH 7.5 +	sporulation occurs but the size of spores is reduced

Varicosporium elodeae:

pH 5.0 - 6.5	optimum for sporulation
pH 6.5 - 7.0	tolerated - sporulation still occurs

Tetracladium marchalianum

pH 6.5 - 8.0	optimum for sporulation
pH 4.0 - 9.0	total range over which sporulation occurs

Although specific conclusions can be drawn from these results - for instance the lower pH values associated with V. elodeae tie in well with the appearance of its spores only in the more acid river system - it would be unwise to extrapolate them too far. It is interesting to note in this respect that the reduced sized spores of Articulospora tetracladia were produced at the 'unfavourable' pH 7.0 in the buffered water of one river, and at pH 8.0 in the water of another. Thus the situation is not straightforward even within a single species.

In the 'reciprocal' experiments carried out with unbuffered river water, two species A. tetracladia and Geniculospora inflata retrieved from the "harmonic" system were found to produce more spores when incubated in the Dartmoor water, even though the G. inflata spore has never been retrieved from that system. Also interesting is the fact that V. elodeae, associated with dystrophic lakes by Susuki and Nimura and with dystrophic flowing water in this study, was retrieved on submerged leaves from the "harmonic" river; its spores were not found.

This investigation seems to raise more problems than it solves and

caution is obviously needed in interpreting and extrapolating the results.

pH, also temperature and aeration are important factors in the selection of suitable culture conditions for aquatic Hyphomycetes in the laboratory. For instance, Thorton investigated all three of these before attempting his study of growth on different nutrient media. His findings are discussed below (2.2.2.3).

2.2.2.2 The effects of temperature

As with pH, laboratory investigations into the temperature ranges and optima for growth and sporulation of aquatic Hyphomycetes provide useful information on the manipulation of these fungi in the laboratory and throw some light on their role and importance in the field.

Aquatic Hyphomycetes become extremely abundant in tree lined streams in autumn and early winter. Obviously this is the time of maximum leaf input but this cannot be the only factor: temperature appears to be important here. In the colder months the enormous aquatic Hyphomycete spore load is accompanied by up to 5 species of these fungi growing on every leaf. The activity of other fungi and bacteria is minimal compared to this overwhelming presence. The conclusion was that these fungi could thrive more readily than their potential microbial competitors at this time and at these temperatures. Thus Thorton introduced his study of temperature with the statement "If aquatic Hyphomycetes can metabolize normally at moderately low temperatures, they may be able to compete ecologically with other less well adapted fungi". (1963). His experiments were designed both to test this assumption and to provide useful information on optimum conditions for his nutritional studies. He compared the growth of 8 species of aquatic Hyphomycete over the range 5°C to 25°C (in 5°C stages). The optimum temperature

lay between 20 and 25°C for 6 of these. He compared these optima with those for certain other lower aquatic fungi; these were "found to be somewhat in excess of those of the aquatic Hyphomycetes".

Since reproductive ability also contributes to the dominance of a fungus in its environment, he investigated the effect of temperature on sporulation. For the two species studied - A tetracladia and V. elodeae there was an apparent preference for 15°C. Perhaps more important, ecologically speaking, sporulation was good at 10°C and still occurred at temperatures as low as 5°C.

Nilsson gives an account of some experiments of his own, as well as a summary of previous work. He makes some general comments on sporulation. Leaves retrieved from streams and showing little or no sporulation can be induced to produce spores after only a few hours when incubated in water at room temperature. Rich sporulation occurs after about 24 hours but this falls off considerably after a few days. Under more 'natural' conditions - that is at stream temperatures - spore production is less profuse but continues over a much longer time. Thus the fungi 'pace' themselves under field conditions and are capable of prolonged sporulation. At relatively high temperatures (above room temperature), and when oxygen levels are rather low, spore production ceases and the aquatic Hyphomycetes become overgrown with other organisms (fungi, bacteria and protozoans). He also comments that "the temperature range for growth and sporulation seems to be more narrow than that shown by other authors for common terrestrial fungi".

He reports Tubaki's experiments (1957) where growth was good at 20°C but sporulation failed between 25°C and 30°C. His own experiments showed good growth at low temperatures, with optima below 20°C in nutrient solution and above 20°C on agar, with a decline in growth at 25°C. Optimum

temperatures for sporulation were between 15°C and 20°C. Thus Nilsson's and Thorton's accounts agree very well.

More recent studies on temperature have been carried out on aquatic Hyphomycetes retrieved from terrestrial sources. Koske and Duncan (1974) compared the optimal mycelial growth temperatures of their "terrestrial" isolates (12 species found on leaf litter) with those obtained by other workers for "aquatic" isolates. They pointed out the very seasonal occurrence of many aquatic Hyphomycetes (particularly those with tri- and tetraradiate conidia) and suggested temperature as a critical factor. In their study of germination, 100% success occurred in species at 5°C: an ecologically significant observation. The species which showed a low and narrow temperature range were found to have a more strictly seasonal occurrence or a more markedly seasonal abundance. For example, Gyoerfyella craginiformis, found sporulating abundantly on decaying leaves in winter and spring, but rarely in summer, produced spores in very low numbers at temperatures over 10°C.

Singh and Musa (1977) studied the effects of temperature on growth, sporulation and spore germination. Their laboratory studies showed higher optima, about 25°C for sporulation. They concluded that this was related to their terrestrial occurrence, linking their observations to those of Koske and Duncan (Nilsson also recorded higher optima for those grown on solid versus liquid media). Perhaps it might be more logical to associate the higher optima with tropical rather than terrestrial conditions.

A quotation from Bärlocher and Kendrick (The Dynamics of the Fungal Population on Leaves in a Stream: 1974) provides a comment on this point and a useful summing-up: "...the main factor which decided which fungi would be dominant in the early succession on the leaves used in the present study

in Ontario seems to have been ability to grow at very low temperatures. But aquatic Hyphomycetes also occur in the tropics, so it is unlikely that it was primarily cold which led to their obvious success in well aerated streams around the world."

2.2.2.3 The effects of aeration

The effects of aeration are considered below, along with other factors that affect sporulation specifically. However a brief consideration of the effects of aeration on growth, and the importance of these observations to laboratory culture and ecological understanding, are given here.

Nilsson (N64) pointed out that aquatic Hyphomycetes could only be kept growing and sporulating in shallow dishes and other containers for a few days. However, mycelia on leaves or growing on agar, if submerged in water through which air was bubbled, carried on growing and sporulating for many weeks. He added a caution however - some species of aquatic Hyphomycete can grow in quite anaerobic conditions. Thus Tubaki (1957) found that Articulospora tetracladia (a good example of such a 'versatile' species - see section 2.1) grew almost as well in anaerobic conditions as it did in aerobic. This result confirms an ecological exception rather than providing an awkward anomaly. Another ecologically interesting observation was the way in which well-aerated conditions limited the growth of lower aquatic fungi.

Thornton's thesis is notable for the careful way in which each problem is approached and each experiment designed. He was anxious to provide suitable, optimal conditions of growth for the aquatic Hyphomycetes whose physiology he was studying. This involved pilot studies on the influence of aeration - as well as pH and temperature - and allowed more confidence to be placed in his determinations of the amount of growth exhibited by the fungi under different nutritional regimes.

During a brief study on the optimum pH for growth in two species of aquatic Hyphomycete he found that the liquid medium used, whose pH was initially well within the optimum range, became progressively more acid due

to fungal respiration. This led to inhibition of growth. In those experiments where a buffer was included to maintain pH growth was also affected, this time due to inhibition by the buffer itself. An awkward problem; however the experiments on aeration provided a solution.

Monod had come to the conclusion that aeration was essential in quantitative bacterial work. Thorton points this out and remarks on the amount of quantitative work done on fungi where this important cultural influence has been ignored. In his nutritional studies conclusions were to be based on the weight of washed mycelium produced. He therefore set up a pilot study where the growth of mycelium in his basic liquid medium was compared under two conditions; when the culture flasks were left standing (air could enter); and when they were forcibly aerated by continuous shaking. The shaken cultures reached a higher maximum weight than those that had been left standing. He attributed this both to increased aeration and to a rise in pH - to be more accurate a lack of fall in pH. Apparently in the shaken cultures the acid produced on respiration formed a usable carbon source. Shaken cultures left to stand showed a fall in pH, whilst standing cultures subjected to aeration showed a further increase in the weight of mycelium produced. This type of study is extremely useful in elucidating suitable conditions for both the study and maintenance of fungi under laboratory conditions.

2.2.2.4 Various influences on sporulation

Any studies on those factors which affect sporulation in the aquatic Hyphomycetes - which inhibit, cause or enhance it - will be of particular value to those studying the ecology of these fungi. The effects of pH and temperature have already been discussed. Three main topics will be

covered in this account:

1. The inseparable and combined effects of aeration and turbulence, mirroring conditions in a fast flowing stream.

2. The reaction of these fungi to various types of damage, some important only under laboratory conditions, some more important in the field.

3. The ability of these fungi to sporulate when not submerged under water; for instance, when growing on solid media.

These three topics overlap to some extent.

The effects of aeration have already been touched on. The aeration of liquid cultures, or of water containing agar pieces or leaves bearing aquatic Hyphomycetes, has the advantage of prolonging the vegetative and reproductive life of the fungus; it may also promote more vigorous growth. However, forced aeration has two "components": it creates turbulence as well as increasing the amount of oxygen dissolved. These two effects also occur in those streams where the characteristic spores of aquatic Hyphomycetes are found so abundantly.

Webster and Towfik (1972) decided to study these effects quantitatively. Discs of agar cut from colonies of aquatic Hyphomycetes, or mycelial pellets produced in shaken culture, were put in water and then forcibly aerated by two methods: the air was passed as small bubbles through a sintered glass bulb; or as large bubbles through a hypodermic needle.

They found that the number of spores produced varied with the rate of aeration. They then extended their investigations to find out which of the effects associated with forcible aeration stimulated spore production. Oxygen concentration did increase a little as aeration rate increased but this could not explain the increase in spore numbers. An experiment using nitrogen instead of oxygen was almost as successful in stimulating spore

production. Could turbulence have stimulated sporulation? The more violent agitation of the cultures produced by the large bubbles of the hypodermic was more effective at stimulating spore production than the gentler action of the glass aerator at the same aeration rate - even though these small bubbles produce a higher concentration of dissolved oxygen.

Perhaps aeration removes gaseous growth inhibitors. The effects of 'fresh' and recirculated oxygen were compared. There was no significant difference in spore production under the two different regimes.

Could aeration affect translocation of nutrients? Experiments using washed mycelial pellets instead of agar discs gave essentially the same results - an increase in spore production with aeration rate.

To investigate the effects of turbulence further, cultures were placed on a special magnetic stirrer. To distinguish the mechanical effects of agitation from those of aeration the cultures were either stirred at a constant rate whilst aeration rate was varied; or stirred at different rates whilst aeration was kept constant. Stirred cultures always produced more spores than unstirred and sporulation increased with increasing stirring at a constant aeration rate. They suggest that this response to mechanical agitation may be an adaptation to turbulent stream conditions.

The enhanced production of spores as water flows over a rough stream bed, or down a slope, or over a waterfall should be taken into account when using the number of spores retrieved as an indication of the abundance of the fungi on their substrates. It may be that even heavy rain can have such an effect; though rain may contribute in other ways to the spore load (see Section 2.3.6.2).

Webster (1975) continued experiments on aeration, examining several reasonable hypotheses for the effect of turbulence in increasing spore

production. He found that increase in forced aeration rate could accelerate spore production in some species. This was not due, with one exception, to a premature detachment of smaller, or undeveloped spores. In V. elodeae the variable spore form had progressively fewer arms as aeration rate increased.

The production of shorter conidiophores - and presumably more rapid initiation of spore production - was demonstrated for one easily studied species only (Lemonniera aquatica).

Perhaps increased aeration induced more conidiophores to develop per unit area of culture disc? Webster found significant increases in density at higher aeration rates. "This idea is also supported by the finding that many fungi grown under conditions of submerged fermentation branch more profusely if vigorously stirred. Since in aquatic Hyphomycetes submerged apices of vegetative hyphae appear to be transformed directly into conidiophores, any treatment which increases the frequency of branching would also increase the amount of sporulation". Further evidence was provided by the continued production of large numbers of conidia when the aeration rate was dropped from high to low. This is presumably the major effect of aeration, and turbulence, on the sporulation behaviour of aquatic Hyphomycetes.

Aging cultures do not sporulate as vigorously as younger cultures. Younis (1966) Nilsson (N64) and Iqbal (1972) found that cutting and wounding old aquatic Hyphomycete cultures promoted and enhanced sporulation. Sporulation occurred principally along the cut edge itself; this phenomenon has been observed in cultures of other types of fungi. When leaves bearing vegetative growth of aquatic Hyphomycetes are cut up and submerged in water sporulation occurs more profusely along the cut edge than on the leaf margin

itself. Cutting can therefore be of great use when large numbers of spores are to be produced and can accelerate the identification of fungi growing only vegetatively on cultures and on substrates.

Iqbal investigated the effect of u-v irradiation in stimulating sporulation. He showed that the effect was suppressed vegetative growth followed by the development of most hyphae as conidiophores. This must be the effect of cutting: vegetative growth is physically arrested and the damaged hyphae grow on as conidiophores, just as submerged vegetative hyphal apices switch to conidiophore development.

When Ingold (1942) grew colonies of aquatic Hyphomycetes on malt agar he found it necessary to submerge pieces of the colony in water before sporulation occurred. This procedure has been followed by other people; sometimes they have confirmed the need for special treatments to induce sporulation. However, several other investigators, principally those studying terrestrial isolates of aquatic Hyphomycetes, have questioned this need. They explain their success in producing sporulation on dry, solid media in several ways.

Nilsson (N64) found difficulty in producing spores on nutrient agars and had to resort to submergence. When aerial sporulation did occur the spores were often not typical "aquaspores" but aberrant or aerial forms. Abundant production of aerial spores reflected the more versatile nature of some of the species. However, on plain agar, cut into pieces but not submerged, sporulation was almost as profuse as on submerged pieces. Also, pieces of colony placed in distilled water produced spores faster and more abundantly than lake or tap water, or nutrient solutions. His conclusion was that too rich a medium prevented or restricted sporulation (vegetative growth is encouraged at the expense of reproductive growth). Also that

cutting further stimulated sporulation (as above).

Bandoni (1972) and Koske and Duncan (1974), growing terrestrial isolates of aquatic Hyphomycetes on solid MYP agar, had little difficulty in producing typical spores. They contrasted ease of sporulation on this medium with a much poorer performance on malt and oat agars - traditional culture media which adequately support vegetative growth - and concluded that the medium used was a critical factor. However, the fact that these particular fungi had been isolated from terrestrial habitats might suggest that different strains of the various species were involved. Singh and Musa (1977) found that their terrestrial isolates of tropical aquatic Hyphomycetes sporulated even on relatively rich malt agar. They concluded that this was due to their terrestrial origins.

Further work will no doubt elucidate the difference between culture media, culture conditions and the origins and "lifestyle" of the fungi involved.

2.2.3 The substrate

Aquatic Hyphomycetes are typically found on submerged leaves of deciduous trees. In elucidating their ecological role, work on their seasonal occurrence in their typical stream habitat can be coupled with knowledge of leaf composition (and changes in composition with decay), and information on their nutritional requirements and capabilities. Recently quite a lot of work has been done on the seasonal and successional behaviour aquatic Hyphomycetes (eg. Triska 1970, Iqbal 1972, Bärlocher and Kendrick 1974, Suberkropp and Klug 1976). Knowledge of leaf composition is quite extensive: this is especially so for terrestrial leaf litter, but work has also been done on submerged plant material (eg. Newton 1971, Suberkropp et al 1976; see section 3.2.3). However, laboratory work on nutritional

requirements, except for information on suitable laboratory culture conditions, is restricted almost entirely to the work of Nilsson (N64) and Thornton (1962, 1963).

In this brief account the following main topics will be discussed: the range and characteristics of media which will support aquatic Hyphomycetes in the laboratory; experiments on their ability to use important carbon and nitrogen sources; and their requirements for vitamins.

Many observations have been made on the media capable of supporting the growth of aqautic Hyphomycetes in the laboratory (see section 2.2.2.4). Nilsson points out two important features in this respect. Firstly, growth and sporulation in distilled water and on very weak media (such as plain agar) demonstrate their lack of special growth requirements and the ability to grow on substrates with very little nutrient available. In fact, aquatic Hyphomycetes have been shown to grow on glass slides immersed in streams (Sladeckova 1963) - though here dissolved nutrients are available to them (see below). Secondly, aquatic Hyphomycetes grow well on a variety of common laboratory media (though sporulation may require special conditions): this is so even in "synthetic nutrient solutions containing only minerals and minute amounts of carbon."

2.2.3.1 The carbon nutrition of aquatic Hyphomycetes

The ability of aquatic Hyphomycetes to grow on various carbon sources is an important and interesting field of investigation. Thornton preceeded his laboratory studies on carbon nutrition with analysis of the different carbohydrates found in the leached leaves of several tree species. This gave him a list of ' ecologically significant' carbon substrates. The growth of 8 different species of aquatic Hyphomycetes and 3 species of common water

mould (aquatic Phycomycetes) was measured on the following 8 sources of carbohydrates: glucose, fructose, maltose, cellobiose, xylose, starch, sucrose and methylcellulose. Briefly all the aquatic Hyphomycetes grew on all the carbohydrates substrates provided-except that 2 species showed no growth on starch and, more importantly, none showed any growth on methylcellulose. The 3 species of aquatic Phycomycete showed growth on glucose only. (see Table 2.1) Thus aquatic Hyphomycetes are capable of using a variety of carbohydrates, all found in leaves. This must contribute greatly to their sustained growth and dominance on leaf substrates.

Nilsson augmented observations on cellulose use by the sucessful growth of 2 species of aquatic Hyphomycete (Tetracladium marchalianum and Margaritospora aquatica) on a medium containing inorganic salts and filter paper asthe sole carbon source. He pointed out that no "far-reaching conclusions" could be drawn from this work, but commented: " I am convinced that the majority of the true fresh water Hyphomycetes take an active part in the decomposition of cellulose and other organic material."

Bärlocher and Kendrick (1974) remarked on the lack of information on the enzymic capabilities of aquatic Hyphomycetes. They stressed that "vegetable materials usually contain high proportions of lignin and cellulose, two substances which are decomposed slowly, if at all, by many organisms." There is quite a lot of circumstantial evidence for cellulytic ability in these fungi. Many field and laboratory studies have observed them growing on well-decomposed leaves with very little in the way of carbohydrate except cellulose. Since then Park (1974) has observed that the versatile terrestrial and aquatic fungus Tricladium terrestre could clear cellulose agar but no quantitative measurements were made and this was the only species under study. In Bärlocher and Kendricks' review article (Gareth-Jones 1976) on the role

The molar growth yields of some aquatic Hyphomycetes on various carbon sources

Species	Carbohydrate						
	Glucose	Fructose	Sucrose	Maltose	Xylose	Cellobiose	Starch
Articulospora tetracladia	81·7*	83·0	85·6	68·0	80·0	62·1	81·3
Flagellospora penicillioides	72·2	68·4	94·4	84·0	78·2	89·9	—
Heliscus lugdunensis	85·0	97·0	111·5	89·0	79·6	89·5	73·4
Tricladium angulatum	97·0	107·0	80·1	87·5	81·6	104·5	87·5
T. splendens	99·5	96·6	111·5	86·5	89·4	94·0	83·0
Tetracladium setigerum	87·5	86·0	87·3	84·0	78·2	80·9	59·7
Varicosporium elodeae	79·4	82·4	91·0	80·5	69·6	89·4	82·9
Volucrispora aurantiaca	87·4	89·9	91·0	94·8	95·5	107·0	—
Pythium ultimum	84·1	—	—	—	—	—	—
Saprolegnia litoralis	93·0	—	—	—	—	—	—
Dictyuchus sterile	49·4	—	—	—	—	—	—
Sordaria fimicola	83·5	—	—	—	—	—	—
Merulius lacrymans	72·4	—	—	—	—	—	—

* Molar growth yield is expressed as mg. dry wt. mycelium produced per millimole of carbohydrate utilized. Carbohydrate was supplied to above cultures at a concentration of 4 g./l. medium. Incubation was in shaken culture at 15°.

The yields (mg.) of aquatic Hyphomycetes on nitrate and ammonium ions

Species	14 days incubation yield/flask, nitrate	11 days incubation yield/flask, ammonium	Controls (no nitrogen)
Articulospora tetracladia	28·6	31·7	1·6
Varicosporium elodeae	29·4	33·8	1·4
Tricladium splendens	33·7	38·0	1·7
Flagellospora penicillioides	29·5	28·9	1·8
T. angulatum	22·3	26·3	0·6
Heliscus lugdunensis	31·7	36·2	0
Volucrispora aurantiaca	Trace	0	0

Percentage utilization of nitrate and ammonium ions by aquatic Hyphomycetes on the 6th and 13th days of incubation at a temperature of 15°

The medium contained glucose, 4·0 g.; KH_2PO_4, 1·0 g.; $MgSO_4$, 0·2 g.; $FeCl_3$, 0·02 g.; biotin 5 μg.; pantothenic acid, 5 μg.; distilled water, 1 l.

Species	6th day		13th day	
	% NH_4 utilized	% NO_3 utilized	% NH_4 utilized	% NO_3 utilized
Articulospora tetracladia	99·4	67·4	99·4	96·1
Varicosporium elodeae	82·3	21·0	99·4	98·0
Tricladium splendens	74·1	22·4	99·4	98·9
T. angulatum	84·9	9·8	99·4	98·4
Flagellospora penicillioides	79·1	26·4	99·4	99·4
Heliscus lugdunensis	65·2	14·5	97·5	99·4

Table 2·1 From Thornton 1963.

of aquatic Hyphomycetes as intermediates in the decomposition of leaf material by invertebrates two important points are made. They stress the superior ability of fungi over bacteria to degrade cellulose and lignin - substances that cannot be digested by most invertebrates. They also point out the universal occurrence of aquatic Hyphomycetes on decaying leaves in streams all over the world. Suberkropp and Klug in their interesting and comprehensive study "Fungi and bacteria associated with leaves during processing in a woodland stream" (1976) discuss successional and seasonal patterns of aquatic Hyphomycete occurrence on leaves. In their concluding remarks they state "Preliminary data from our laboratory indicate that all of the aquatic Hyphomycetes are capable of elaborating pectinase and cellulase". This would mean that these fungi are capable of macerating the leaf tissue as well as breaking down its major structural component. Unfortunately the results of this important work are not yet available.

2.2.3.2 The nitrogen requirements of aquatic Hyphomycetes.

All living organisms require nitrogen: many lower fungi, including most aquatic Phycomycetes, require this nitrogen "ready formed" as organic compounds. Thorton investigated the ability of aquatic Hyphomycetes to use the inorganic nitrogen available from solution in stream water. The growth of seven species was examined on the two most usual sources of dissolved nitrogen in streams: ammonium and nitrate ions. All but one species grew on both sources. A slight preference for the ammonium ion was observed. This preferential use was confirmed by % utilization using a mixture of ammonium and nitrate. Thornton suggested that "aquatic Hyphomycetes may benefit more from the nitrate and ammonium ions leached from the soil into streams than do other aquatic molds". (See Table 2.1).

It may be appropriate here to mention some field and laboratory investigations into fungal decomposition and the nitrogen content of decomposing leaves. Kaushik and Hynes (1971) immersed leaf discs (of various species) in a river. The retrieved discs showed degradation, mainly by fungi, accompanied by loss of weight but an increase in nitrogen content. This was attributed to the ability of the fungi to use soluble nitrogen sources for incorporation into fungal biomass, rather than decreasing the nitrogen content of the leaf by withdrawing and using ready formed organic sources. They followed up these field observations with a series of investigations in the laboratory where leaves were bathed in solutions enriched and unenriched with additional nitrogen and phosphorus. The increase in nitrogen was greater in enriched solution. Antifungal and antibacterial substances were included, separately and in combination, to assess the contribution made by the fungal and bacterial leaf floras. The results confirmed the role of the fungi; no nitrogen increase was observed when antifungal substances were included. Kaushik and Hynes decided that terrestrial Hyphomycetes were important members of their leaf floras - though the incubation conditions used may have restricted the species of fungi "discovered" (see section 4.1 and Suberkropp and Klug 1976).

Newton (1971) found that whole Alnus leaves, placed in a river and then retrieved at intervals for chemical and mycological investigation showed progressive increases in protein content. This appears to be a reflection of the same phenomenon. She found that both aquatic phycomycetes and aquatic Hyphomycetes were important members of the leaf mycoflora.

It seems reasonable, taking into account the particular ability of aquatic Hyphomycetes to use soluble inorganic nitrogen, to extrapolate the principle of nitrogen'trapping' and incorporation to this group of fungi.

However the results obtained in these field and laboratory investigations inevitably reflect the methods chosen and the particular river and leaves studied.

2.2.3.3. The vitamin requirements of aquatic Hyphomycetes

Aquatic phycomycetes often have a special growth requirement for vitamins. The vitamin requirements of aquatic Hyphomycetes were studied by both Thorton and Nilsson. Thorton tested various B vitamins and vitamin C. "several species of aquatic Hyphomycete had no detectable vitamin requirements, whilst in others the lag period of growth was shortened in the presence of certain vitamins...." Nilsson looked at the effects of vitamins on the growth of two species (T. marchalianum and M. aquatica). Both could grow without these vitamins but growth was improved in some of the vitamin enriched media.

Sparrow summarises the nutritional ability of aquatic Hyphomycetes in his review article on the ecology of various freshwater fungi (Ainsworth and Sussman 1968) ".... the ease with which these hyphomycetes utilise inorganic nitrogen sources gives them an advantage over the more fastidious water molds.... also ....imperfects use a wide range of simple and polymeric carbohydrates and again have a nutritive advantage over their competitors, the Saprolegniaceae, which are more restricted in their carbon sources". The importance of cellulytic ability to saprophytic fungi such as aquatic Hyphomycetes, the circumstantial evidence pointing to this ability, but the lack of laboratory investigations into this ability, have all been stressed above.

2.3 The Ecology of Aquatic Hyphomycetes

2.3.1 Introduction

Aquatic Hyphomycetes have been studied principally in the well-aerated streams of temperate areas. In such streams the main substrate is the seasonal supply of deciduous tree leaves. This emphasis is reflected in those major studies which consider the ecological role of aquatic Hyphomycetes, for example: "Seasonal distribution of aquatic Hyphomycetes in relation to the disappearance of leaf litter from a woodland stream" (Triska 1970); "Fungi and bacteria associated with leaves during processing in a woodland stream" (Suberkropp and Klug 1976) ; "Dynamics of the fungal population on leaves in a stream" (Bärlocher and Kendrick 1974).

It is probably true to say that the world-wide importance of aquatic Hyphomycetes is accurately based on their role as decomposers of leaf material in such waters: these are the habitats and substrates in which the abundance and variety of aquatic Hyphomycete species has rightly attracted the attention of ecologists and mycologists.

Because of this emphasis, quite a lot is known about the decomposition of several different species of tree leaves aided by known species of aquatic Hyphomycete. However the importance of these fungi, even taken as a group, may not be adequately appreciated on other substrates, in other geographical locations. Much, of course, is and can be inferred.

The autecology of aquatic Hyphomycetes is discussed below under several convenient headings. Inevitably however these accounts inter-relate and overlap, and summary patterns are included to help draw the threads together.

2.3.2 Substrates for aquatic Hyphomycetes

Aquatic Hyphomycetes have been found growing on a variety of natural and

artificial substrates both in water and on land.

Table 2.2

Substrates of aquatic Hyphomycetes

substrate:	references:
In water	
Natural substrates	
Deciduous tree leaves	numerous
Herbaceous dicot. leaves	eg. Padgett 1976 (tropical)
Grass culms and leaves	eg. Iqbal 1972
Coniferous needles	eg. Bärlocher and Oertli 1978
Wood	several - eg. Willoughby & Archer 1973
Artificial substrates	
Glass slides	Sladeckova 1964
Glass slides coated with rosin	Bärlocher et al 1977
Plastic	
Paper	
String	reported by Nilsson (N64)
Cloth	
On land (see section 2.3.6.1)	
Deciduous tree leaves	eg. Bandoni 1972
Herbaceous leaves and stems	eg. Bandoni 1972
Grass culms	eg. Webster 1954
Living grass leaves	eg. Makela 1972
Wood	Price and Talbot 1966
Root surface	eg. Waid 1954
Soil	Gams, Domsch and Weber 1969

Some species, for example Tetracladium marchalianum (typically aquatic) and Varicosporium elodeae (versatile) can be found on a great variety of substrates. These tend to be the species with particularly well adapted spores; they are also likely to have a wide geographical distribution. Other are less widely found. For instance Tricladium giganteum has a "particular predilliction for grass" (Willoughby 1975) whilst the enormous spore of Polycladium equiseti "....so far as I know has only been collected once. It was found in abundance on dead bleached submerged internodes of Equi setum fluvitale". (Ingold 1975a). The huge spore Actinospora megalospora appears to be limited to submerged wood (Archer and Willoughby 1969).

In well-aerated streams a newly fallen submerged leaf rapidly loses much of its soluble components. As decomposition progresses the softer parts tend to disappear - aided by stream invertebrates which prefer to eat these parts. This leaves a network of resistent veins. Although aquatic Hyphomycetes appear rapidly after the leaf enters the stream, the variety and abundance is often striking on these skeletonised leaves. Some species do tend to occur more abundantly near the beginning of leaf processing, perhaps exploiting the more readily available leaf components.

Heliscus lugdunensis, on the other hand favours the hard parts of the leaf, its veins and lamina and so its numbers increase as the leaf skeletonizes.

Suberkropp and Klug (1976) suggest that all species have cellulytic ability. This, as well as an ability to use most of the simpler carbon compounds in the leaf and to augment nitrogen supplies from the surrounding stream water, explains the presence of aquatic Hyphomycetes throughout the first stages of leaf processing—from newly shed leaf to part of the large pool of detrital particles.

The balance of species and abundance of these fungi does change during the decay of a particular species, though this may be due to environmental factors as well as chemical changes in the substrate. Different species of leaf examined at the same time in the same stream for Hyphomycete growth will not necessarily be colonized by the same species or to the same extent. For instance Bärlocher and Kendrick (1974) found that Tricladium angulatum was an abundant member of the maple and ash mycofloras, but was rarely found on oak. Some species, and these are usually the ones which decompose rather rapidly, nearly always show abundant growth. Alder is a good example. The aquatic Hyphomycetes described in Ingold's first study of 1942 were found on alder leaves. Leaves such as oak or beech decompose more slowly than other species, probably due to their tough leathery texture and the inhibitory effect of the tannins they contain. Aquatic Hyphomycetes therefore take longer to build up on these slow decomposers and may never show the variety and abundance exhibited on other leaf species.

It is difficult to generalize about individual species of aquatic Hyphomycete. A different combination of leaf species and stream conditions may alter the detailed seasonal and successional appearance of the species involved. But as a group they are conspicuous colonizers of deciduous leaves and their presence as spores and colonizing hyphae in early winter can be massive.

Until recently it was thought that aquatic Hyphomycetes did not grow on needles from coniferous trees, and therefore could not contribute to their decomposition. Bärlocher and Oertli, however have recently shown "that they occur though at low frequencies on the needles of at least one conifer Pinus resinosa". Experiment showed that these low numbers were due to inhibitory substances rather than lack of nutrients.

Records of aquatic Hyphomycetes growing on wood are reasonably common. A few are found almost exclusively on wood but for many it appears to provide an 'alternative substrate' in addition to leaves (see 2.3.4.2). This is especially so with those imperfect species which have a perfect stage that grows on wood, such as Heliscus lugdunensis and Anguillospora crassa. Gareth-Jones (1976) lists 15 aquatic Hyphomycetes as 'fresh-water lignicolous fungi' in his summary of all freshwater fungal species found on wood. This includes such well known species as Alatospora acuminanta, Articulospora tetracladia, Clavariopsis aquatica, Lemmoniera aquatica and Tricladium splendens. Growth on wood, of course does not necessarily imply an ability to use lignin.

Willoughby and Archer (1973) provide a major contribution to knowledge of the aquatic Hyphomycete flora of twigs in "The fungal spora of a fresh-water stream and its colonization pattern on wood". They found H. lugdunensis - common on the harder parts of leaves - to be an early colonist of submerged twigs whilst Dimorphosphora foliicola was a late colonist. Experiment and observation suggested that the sugar reserves in twigs stimulated the initial colonization by H. lugdunensis; whilst the continued presence and slow release of such sugar might "prolong and complicate the pattern of succession on twigs". Leaves lose most of their leachable sugars in the first few days of immersion in water (Kaushik and Hynes 1971).

Iqbal found Varicocosporium elodeae particularly common on submerged grass in Dartmoor rivers. This explained the high frequency of V. elodeae spores in a moorland area where grass probably provided more vegetable material for colonization than did tree leaves.

The presence of aquatic Hyphomycetes growing on such inert structures as glass and plastic reflects their ability to 'trap' nutrients from the surrounding water whilst using these 'substrates' purely as a hold-fast (Bärlocher and Kendrick 1974).

2.3.3 Habitats of aquatic Hyphomycetes

Taken as a group: "A well aerated stream, lined with trees and shrubs of all kinds, especially alder, hawthorn or oak, provides an ideal habitat for aquatic Hyphomycetes". (Iqbal 1972 - my emphasis). This type of 'stock' description of the typical habitat and substrate of these fungi is re-iterated in numerous papers. However, due to the success of the group as a whole, and to certain versatile species in particular, these fungi are also found in a wide variety of still and running waters, as well as on land. The range of habitats from which aquatic Hyphomycetes have been recorded is set out below.

Table 2.3

Habitats of aquatic Hyphomycetes

Aquatic habitats		
Streams		numerous references
Rivers		numerous, eg. Iqbal and Webster 1973
Lakes and Ponds		eg. Suzuki and Nimura 1963
Estuaries		Kirk 1969
Temporary pools		eg. Bärlocher et al 1978
Snow	as spores	Tubaki 196
Foam and scum	as spores	eg. Dudka 1975
Terrestrial habitats		
Soil		eg. Gams et al 1969
Leaf litter layer		numerous eg. Bandoni 1972
Living plants		eg. Makela 1972

Obviously not all of these habitats support an equally varied and abundant aquatic Hyphomycete flora. There is also the problem of whether the fungi have been recorded as colonizing mycelia, or whether they have been found as spores. The differences can be very important. Some lakes, for instance, yield quite a rich spora but Nilsson's view is that "the occurrence of the fungi in large streams and lakes is mainly a result of transportation of the fungi and/or their spores into these waters", and not the record of an indigenous flora.

Rivers and lakes - depending on leaf input and environment - may be quite important habitats, however the abundance and variety of species diminishes in these and other'non-stream'environments. Thus Nilsson (N64) reporting on Swedish findings states that "many species do occur in lakes and ponds, but rarely in abundance" and gives an interesting example of a lake where only one species - Tetracladium marchalianum - "grew on almost every leaf near the shore in a locality exposed to hard wave action". Turbulence seems to have contributed to abundance in this case. He also notes that the occurancc of aquatic Hyphomycetes tends to be irregular in lakes and large streams whilst the majority of species found in smaller streams occur year after year (and often all year round).

Ponds and other small, more or less stagnant waters do yield several species. Few are "completely aquatic species" but tend to include versatiles such as V. elodeae and A. tetracladia. These, along with Margaritospora aquatica and Dimorphospora foliicola are also found in the temporary forest pools which dry up every summer, in temporary streams, and in flooded areas. Each of these species is capable of producing a different form of spore depending on conditions (see 2.1.4).

Nilsson makes a particularly interesting point with respect to the successful colonization of leaves in any aquatic habitat. Aquatic Hyphomycetes are rare or absent in any environment which encourages the heavy growth of algae, phycomycetes and protozoans, or where a layer of mud builds up. Competition for space and substrate is too great for the slow-growing fungi. Thus when he lists those features which make streams "....especially small turbulent ones".... particularly suitable habitats for aquatic Hyphomycetes he includes the cleaning action of the stream water.

Although spores of aquatic Hyphomycetes are washed down to estuaries they are unlikely to germinate and grow successfully; growth seems to decrease with increase in salinity (N64). Kirk (1969) however has found aquatic Hyphomycetes growing on wood in an estuary.

Tubaki (1963) and others have found the spores of certain aquatic Hyphomycetes in snow - including A. acuminata, A. longissima and T. angulatum - along with other branched propagules whose fungal or algal identity is uncertain.

Dudka and Beregovaya (1975) write of foam and film as an 'ecological niche' of aquatic Hyphomycetes in which the viability of their conidia is preserved and in which they can be dispersed (see 2.3.5).

Aquatic Hyphomycetes have been recorded, at first occasionally, recently more systematically from terrestrial habitats. This interesting topic is treated separately in section 2.3.6.1.

Nilsson (N64) draws up a table giving the records of each of the Swedish species from a variety of aquatic, semi-aquatic and terrestrial biotopes. This indicates in which biotope each species is generally found. Thus the versatile fungi mentioned above are more commonly associated with stagnant water, semi-aquatic and terrestrial conditions; whilst the well known

tetraradiate and sigmoid forms generally occur in streams. However, some of the ubiquitous species, such as A. acuminata, H. ludunensis and L. aquatica, although associated mainly with a completely submerged existence, have been recorded from a very wide range of habitats. Nilsson remarks that although it is obvious "that some [species] are semi-aquatic and many of them not completely aquatic.....knowledge of the ecology of the group is still too superficial to permit a definite classifying in(to) non-aquatic and aquatic species". Perhaps the interesting feature of this aspect of the ecology of 'aquatic' Hyphomycetes is their 'refusal' to be classified in such a way. Tetraradiate and sigmoid spored species dominate the stream habitat yet may also grow in a variety of other habitats, whilst fungi not as well adapted to a completely submerged existence can thrive in semi-aquatic and terrestrial biotopes, bridging the gap in habitats which switch from aquatic to terrestrial conditions, such as temporary pools and intermittently flooded areas.

Attempts have been made to classify the more important habitats of aquatic Hyphomycetes in order to pin-down the factors which make them suitable. Not all streams, for instance, are equally suitable: and when does a stream become a river?

Classification is not easy. "The physical environment in which the aquatic Hyphomycetes occur presents one of the largest obstacles in the ecological study of these fungi. The fungi are in a constantly changing environment and the source of some of the conidia is uncertain". Many 'unknowns' are involved. Qualitative descriptions of the habitat may be inexact, quantitative measurement may be hard to make, or may relate to factors too local or variable to be of any use in a general classification. Many records are of sporas alone, so that the flora of the habitat is unknown.

For instance, turbulence may increase, and pH decrease the numbers of spores produced within a habitat whilst vegetative growth remains largely unaffected. Runoff from the terrestrial environment and spore loads brought from upstream of a particular site may bias the spora with respect to the flora.

Suzuki and Nimura's classification of lakes, with pH range related to species found, was mentioned in 2.2.

Nilsson gives a comprehensive but qualitative classification of streams, making a particular note of size and physical conditions. He gives an indication of abundance and a list of representative species from each of the stream types. To summarize his observations: "My investigations in Sweden have shown that the main biotope for freshwater hyphomycetes is a small well-aerated oligotrophic to eutrophic forest stream". Little growth occurs in small alpine and subalpine brooks deprived of suitable substrate. Whilst in oligotrophic, well-aerated subalpine streams with a dense bordering vegetation growth may be abundant, but species few. Several categories of well-aerated oligotropic to eutrophic forests streams support an abundant and varied flora; whilst slow running eutrophic lowland streams (often with few bordering trees) and stagnant slow running forest streams show little or no growth.

Many other workers, studying aquatic Hyphomycetes as an ecological group important in the decomposition of allocthonous vegetable material, have contributed valuable physical, chemical and biological data - such as information on dissolved nutrients, pH, temperature and leaf input - accompanied by useful species lists. So many parameters are involved that it is perhaps unrealistic to attempt a comprehensive classification of streams and the aquatic Hyphomycetes they support.

2.3.4 The distribution of aquatic Hyphomycetes.

2.3.4.1 Distribution in space: regional and geographical records

Regional distribution: Aquatic Hyphomycetes may be recorded as colonizers of plant material, or sometimes by the presence of their spores alone.

Different combinations of biological, chemical and physical factors, such as the number of trees bordering a stream, the pH of the water, and turbulence of flow may all lead to local or regional differences in abundance and species composition. These factors obviously influence the colonization of leaves and other material by aquatic Hyphomycetes; they may also have a disproportionate effect on the abundance and relative species composition of the spores found. Spores in foam, scum and water are an easy and widely used way of getting an idea of the flora in the locality from which they were collected. Anomalies and discrepancies may arise unless caution is exercised (see 2.3.6.2). For instance, lakes, lower reaches of streams and rivers, and even estuaries may act as 'sinks' for spores washed downstream. As Nilsson comments...."a large number of spores of hyphomycetes are obviously transported into many lakes by the surrounding streams.... the transport from the surrounding system must always be taken into account in discussions on the composition of the microflora of lakes".

Iqbal provides interesting evidence of regional differences (1972). He contends that each river system has its own characteristic spora and contrasts the range of species and dominant species found in a Dartmoor river system and in the River Exe and its tributaries. The use of sporas here seems valid since Iqbal is considering an aggregate spora with contributions from the numerous tributaries which go to make up the system. The sporas seem to reflect the different countryside through which these two system flow. Dartmoor is a harsher upland environment, with fewer trees, and generally

speaking more acid waters, often with humic substances. The River Exe includes lowland tributaries with abundant bordering trees.

Geographical distribution: Nilsson (N64), Petersen (1962 et seq) and Ingold (1966) give a great deal of information on the geographical records and distribution of individual species of aquatic Hyphomycete. Triska, in his 1970 Ph.D. thesis on "Seasonal Distribution of aquatic Hyphomycetes....." also gives quite a comprehensive review of records in an attempt to tie together information from diverse sources on the seasonal occurrence of species. Sometimes the winter or summer appearance of some species is reflected in their major distribution area. Triska found the mainly tropical species Tricelophorus monosporus in summer in his temperate stream, whilst frequent winter recorded species were often exclusive to cold temperate areas.

En masse geographical distribution records are rather overwhelming and useful summaries are also given by Nilsson (N64) and in Ingold's 1976 review paper (in Gareth-Jones).

Nilsson points out that many of the records used to build up the picture of world-wide distribution of the aquatic Hyphomycetes are based on occasional and very local observations. Very few countries boast systematic surveys of their floras. He himself studied several provinces of Sweden: "In well investigated areas... where hundreds of waters have been studied, a certain numbers of species occur in 50-70 per cent of the waters where members of this group are present. Some species such as F. curvula are found in almost every normal type of stream". Such common species, he concludes are likely to be common all over Sweden. They include:

Alatospora acuminata

Anguillospora longissima

Articulospora tetracladia

Clavatospora aquatica

Flagellospora curvula

Lemonniera aquatica

Tetracladium marchalianum

Varicosporium elodeae

These are familiar species from discussions of spore morphology and include the most 'evolved' morphologies of their group, as well as the ubiquitous and versatile V. elodeae. They have also been found to be common members of stream and river floras and sporas from many other temperate areas (see Table 2.6).

However many other species have a very limited or local distribution; this is particularly so for the more peculiar and large spores. Nilsson suggests that these may have special requirements, or they may "have a less well-adapted type of spore and thus have reacted only a limited distribution".

The tropics boast fewer records but appear to support very rich and varied floras. "It is evident from the numerous unidentified spores that the tropics have a rich flora....consisting of many peculiar types sometimes with a tetraradiate organisation, sometimes with quite a different organisation or with only indications of a special adapted spore type" (N64). T. monosporus however represents a successful and well-adapted fungus with a typical tetraradiate shape (Figure 2.7). It is a common and widely distributed species in the tropics. Nilsson concludes: "For some species the climatic conditions ...may be of major importance but for the majority of the truly aquatic species they are not. Some of these are found from biotopes at an altitude of 1000m. in the northern-most regions of the world to extreme tropical lowland biotopes".

He then surveys the known species of aquatic Hyphomycete (in 1964), placing them in one of 3 main groups according to their distributions. His lists are reproduced in Table 2.4)

Table 2.4

Geographical Distribution of Aquatic Hyphomycetes

A. Species with a world-wide distribution, mostly very common. The first group includes species that seem to have their main distribution decidedly in the tropical regions, the second group species with their main distribution in temperate and cold regions:

1.
Actinospora megalospora
Campylospora chaetocladia
Clavatospora tentacula
Flagellospora penicillioides
Heliscus submersus
Triscelophorus monosporus

2.
Alatospora acuminata
Anguillospora crassa
Anguillospora longissima
Articulospora tetracladia
Clavariopsis aquatica
Clavatospora longibrachiata
Dactylella aquatica
Flagellospora curvula
Geniculospora inflata
Lemonniera aquatica
Lemonniera terrestris
Lunulospora curvula
Tetrachaetum elegans
Tetracladium marchalianum
Tetracladium setigerum
Tricladium angulatum
Tricladium gracile
Tricladium splendens

B. Species only found in temperate and cold regions of the world, where they are common or at least distributed in several countries:

Anguillospora pseudolongissima
Casaresia sphagnorum
Clavatospora stellata
Culicidospora aquatica

Culicidospora gravida
Dactylella submersa
Dendrospora erecta
Dimorphospora foliicola
Heliscus lugdunensis
Gyoerfyella craginiformis
Margaritispora aquatica
Monotosporella microaquatica
Tetracladium maxilliformis
Tricellula aquatica
Tricladium anomalum
Varicosporium elodeae

C. Species with a very limited distribution usually only known from the type locality. The first group includes species known only from tropical regions, the second species reported only from temperate and cold regions:

A.
Angulospora aquatica
Centrospora flagellifera
Geniculospora grandis
Jaculispora submersa
Pyramidospora casuarinae

B.
Anguillospora gigantea
Articulospora moniliforma
Bacillispora aquatica
Calcarispora hiemalis
Centrospora angulata
Clavariopsis brachycladia
Clavariopsis bulbosa
Flagellospora prolifera
Flagellospora stricta
Geniculospora intermedia
Lemonniera cornuta
Lemonniera filiformis
Saprochaete ramonissima
Speiropsis irregularis
Tricladium eccentricum
Tricladium patulum
Triscolophorus magnificus
Varicosporium aquaticum

from Nilsson 1964

Bärlocher and Kendrick (1974), discussing the success of aquatic Hyphomycetes in well-aerated streams around the world, conclude that a combination of the ability to sporulate under water with "superior adaptation for attachment to solid surfaces... and other as yet undetermined factors [?cellulytic ability] have probably given this group a competetive advantage from the outset. It seems plausible that they subsequently adapted further to specific qualities of the stream inherent in its geographical location". In this case they have in mind the low stream temperatures and seasonal supply of deciduous leaves found in temperate woodland streams.

Ingold, in his review "The morphology and biology of freshwater fungi excluding Phycomycetes" (1976 in Gareth-Jones), points out that it is only in the aquatic Hyphomycetes group that enough information has accumulated "to allow a tentative picture to be drawn" of world distribution. He suggests that this is due to the ease with which species of aquatic Hyphomycete can be recognized from their conspicuous and easily identified spores trapped in foam and scum. He estimates that 40 genera and 80 species have been described (formally) to date and that these figures may eventually be doubled. He comments "It is almost impossible to get objective information on the relative abundance of biomass of fungi in nature but anyone who studies aquatic Hyphomycetes is soon convinced that they represent the great bulk of fungus life in the well-aerated waters of streams and rivers, if not of lakes and ponds". He gives a useful table of the world distribution of 15 well-known species pointing out the gaps in the records for S. America and Australia. He seems confident that these gaps will be filled and the truly world-wide distribution of aquatic Hyphomycetes confirmed. Bärlocher and Kendrick (1974) make an interesting comment: they point out that few

regions have been systematically examined for aquatic Hyphomycetes and repeat Ainsworth's (1968) contention "that the records almost certainly reflect the activities of interested mycologists and not so much the true geographical distribution of the fungi".

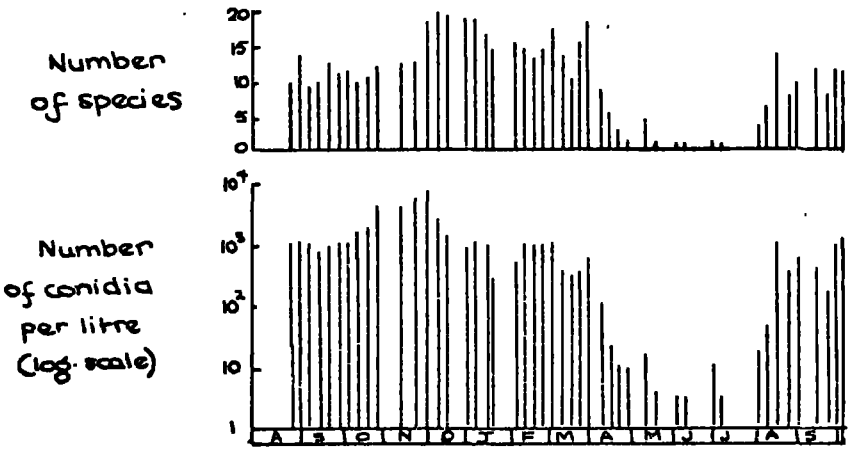
Some of the papers dealing with individual regions and localities make interesting reading. Ingold has produced a large number of papers dealing with species from all over the world. Muira's 1974 survey of Japanese stream sporas gives beautiful and delicate illustrations of 82 different hyaline, thin-walled radiately branched or 3-D elongate spores, only 36 of which belong to known species. Most of the undescribed species come from sub-tropical and tropical sites. Padgett (1976) describing the contribution that aquatic hyphomycetes make to decomposition in a tropical rainforest stream, gives an interesting list of species found in Puerto Rico. This includes such well known 'worldwide' species as Anguillospora longissima, Flagellospora curvula and Tricladium splendens as well as the widely distributed tropical species Tricelophorus monosporus. Lunulospora curvula was a frequently observed fungus in this study; this species is also common in temperate areas but tends to occur in the warmer months of the year (Iqbal and Webster 1973).

2.3.4.2 Distribution in time: seasonal and successional patterns.

Since the typical and most studied substrate of aquatic Hyphomycetes is autumn shed leaves, seasonal patterns of abundance and species diversity are inevitably tied up with successional patterns due to changes in the composition and explotation of leaves. Nearly all investigations have been made in temperate areas where a regular leaf input is coupled with seasonal changes in temperature. Autumn brings leaf fall and a drop in temperature

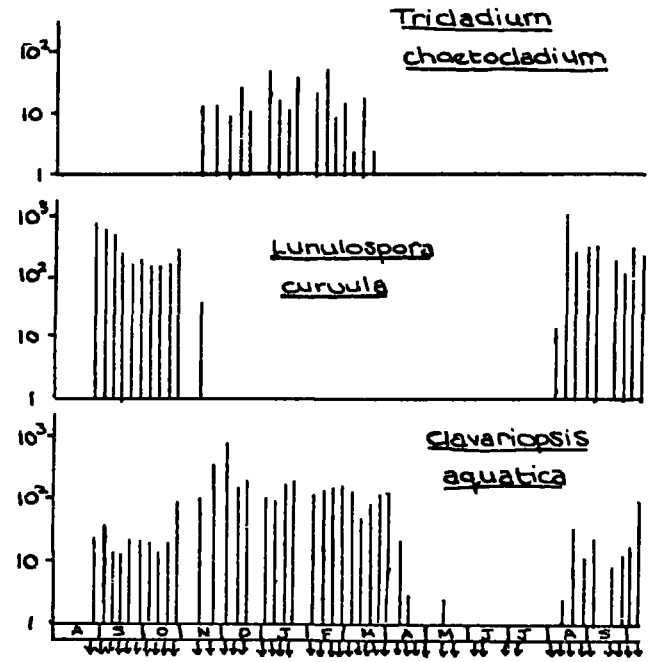
and the abundance and diversity of aquatic Hyphomycetes found on leaves and in foam increases enormously. By summer both the variety and abundance have declined greatly. The initial processing of leaves (from whole leaf to remaining small particles) with which aquatic hyphomycetes are particularly associated may be almost complete in some species. In others a skeleton framework remains, or a brittle tough lamina, much depleted of the nutrients it contained when it entered the stream. The hyphomycete flora changes with these changes in leaf composition.

Aquatic Hyphomycetes seem to fall into three main groups. Those recorded principally in the colder months - 'winter' species; those usually found during the warmer months - 'summer' species; and those found throughout the year, the 'constant' species. Iqbal and Webster (1973b) give clear spore diagrams (illustrated in Figure 2.11) which show the seasonal variations in numbers of spores - Tricladium chaetocladium (winter), Lunulospora curvula (summer) and Clavariopsis aquatica (more or less constant). Variations in the number of species and the total numbers of conidia are illustrated by the same type of diagram. Their data provides a useful example of the type of seasonal variation which occurs in all temperate rivers and streams. Iqbal and Webster comment on the difficulty in interpreting such variations, both within the group as a whole and between individual species. "Explanations for peak periods of sporulation must presumably be sought in terms of substrate availability and the physiology of growth and fruiting of aquatic hyphomycetes in relation to such variables as temperature, pH and competition. Although there have been some studies on the physiology of aquatic hyphomycetes....these studies hardly provide a sufficient basis for explaining the observed differences in spore content of streams throughout the year".



Aquatic Hyphomycetes in R. Creedy, Devon
Each vertical line represents the analysis of a sample taken on the indicated date. Slightly modified after Iqbal 1972.

(from Ingold in Gareth-Jones 1976)



Number of conidia per litre of three aquatic Hyphomycetes in water of R. Creedy, sampled throughout year on dates indicated. Slightly modified after Iqbal 1972

Fig 2.11

This study of seasonal and successional patterns can be rather confusing. Two tables - 2.5 and 2.6 - have been drawn up in an attempt to summarize useful information on the factors which do or may, influence patterns of occurrence (2.5); and on dominant, winter, summer and constant species (2.6 - this table may also be of use in the consideration of spatial distribution, 2.3.4.1; terrestrial occurrence, 2.3.6.1; and in discussion of the field study, 5.3).

Table 2.6 lists several papers in which aquatic Hyphomycetes have been recorded and studied; most are concerned with rivers and streams in temperate countries. The geographical area in which the study or survey was carried out, and the methods used, are indicated. The species listed are those found to be particularly common (unless otherwise indicated). For some of these species information on their seasonal occurrence is available and this is shown. The repeated recording of a handful of species is rather striking. Additional information on successional patterns is included.

Triska's thesis (1970) provides a great deal of information on the temporal distribution of aquatic Hyphomycetes. Not only does he present a great deal of original data on seasonal and successional patterns but also includes a review of other peoples observations for individual species, with additional information on preferred substrates and geographical records. He confirms the well known cycle in species and numbers. He also points out that the "new" leaves which fall off the trees in spring and summer produce a flora which is substantially different from that on older leaves examined at the same time, and which goes against the general seasonal trend, providing interesting information on succession. Lemonniera aquatica and Tetrachaetum elegans are early colonists of both the autumn shed and summer shed leaves

Table 2.5

Factors influencing the temporal distribution of aquatic Hyphomycetes and affecting both abundance and species diversity (in streams and rivers of temperate regions)

ANNUAL CYCLE OF ORGANIC DEBRIS

Availability of leaf substrate: numerous references, eg. Nilsson (N64).

Introduction of new leaves: eg. certain species particularly associated with the new leaves which fall in both autumn and early summer (see Table 2.6) Triska (1970) Suberkropp and Klug (1976).

Chemical Composition of leaf:

Physical condition of leaf: complete or skeletonized, or in small pieces, eg. Suberkropp and Klug (1976): the increase in importance of bacteria as the material becomes fragmented.

RAINFALL

Runoff: introduction of 'terrestrial' spores (see 2.3.6.1) eg. Triska 1970: importance of spring runoff, Willoughby and Archer 1973: increase in spore load after rain, Koske and Duncan 1974: importance of increased terrestrial spore production in winter.

Other factors: heavy rain creates turbulence: agitation of water suspending spores already present; stimulation of spore production by mechanical disturbance. eg. Webster and Towfik (1972)

TEMPERATURE various effects: dominance of aquatic Hyphomycetes over other fungi, at least during the colder months (see section 2.2.2.2 and 3.3); occurrence of 'tropical' species in summer and 'cold temperate' species in winter, etc. eg. Conway 1970, Triska 1970, Bärlocher and Kendrick 1974, Suberkropp and Klug 1974

Triska puts forward leaf availability, leaf composition, physical condition of the substrate, temperature and runoff as important factors influencing the patterns revealed.

For instance, temperature may be an important influence on the appearance of Tricelophorus monosporus, a species usually associated with the tropics. In his study it was found as a 'summer' species and he stresses the 'temperate' or 'tropical' records for each species discussed, relating them to winter and summer appearance.

He noted that the decline in the number of aquatic Hyphomycete species in spring was accompanied by a rise in the number of more 'amphibious' species such as Varicosporium elodeae and aero - aquatic species. He suggests that "spring runoff," when Linesville creek carries its greatest load of sand and soil, is instrumental in the colonisation of leaf substrates by terrestrially oriented fungi and may thus influence the changeover of fungal spores between spring and summer". Runoff carries both terrestrially occurring aquatic Hyphomycetes (Koske and Duncan 1974) and terrestrial fungi proper, mainly in the form of spores. Willoughby and Archer (1973) compare the species retrieved from foam during dry and wet periods. In the dry periods, which occurred mainly in May and June, the spora was exclusively aquatic Hyphomycete. In wet periods, mainly September and October, the spora was much more diverse with both an increase in the number of aquatic Hyphomycete species as well as the presence of versatile hyphomycetes, aero-aquatic hyphomycetes and geofungi. Some of this increase is no doubt due to runoff, though the new leaf fall may have also influenced the species found.

Iqbal and Webster (1973b) used a quantitative spore sampling method (filtration) and concentrated on recording reasonably gross seasonal changes in numbers and species. Although Figure 2.11 shows that no spores were

recorded over part of the summer, parallel leaf collections revealed that some were still growing; the spores produced were at too low a concentration to show up (see 2.3.6.2). Particularly interesting is the 'complimentary' occurrence of Tricladium gracile (winter) and Lunulospora curvula (summer) shown in Figure 2.11. L. curvula is a species particularly associated with warmer countries and warmer months of the year. However, Iqbal and Webster also suggest its preference for alder leaves, which decompose rather rapidly, as a factor in its temporal distribution. The idea that it "is a primary colonizer of leaf material, possibly dependent on soluble materials, cannot be ruled out". They also suggest that L. curvula and T. gracile "would make an interesting pair of species to compare in terms of nutrition, and physiology of growth and sporulation"; such studies might throw some light on such differences in temporal distribution.

Iqbal (1972) suggests that species such as A. longissima, C. aquatica, Clavatospora longibrachiata and T. marchalianum have a more regular distribution over autumn and winter because they can use the simple carbohydrates available in autumn at the start of leaf decay and can then switch to the more resistant substances, such as cellulose, as decomposition progresses.

Suberkropp and Klug (1976) in their excellent study of the microflora of submerged leaves, concentrate on explaining the seasonal changes in numbers and species of aquatic Hyphomycete in terms of their succession on oak and hickory leaves.

They put forward various suggestions for the differences that have been found in such successional patterns, between streams (both inherent differences and perhaps the methods used), and also between leaf species. They stress the lack of physiological information which might help explain

such patterns. Different species may have different enzymatic capabilities though they stress that preliminary evidence suggests that the dominant species found in their study stream are capable of breaking down all the major components of hickory leaves, at least. Thus their enzyme producing abilities will not act as a limiting factor in their all-year-round colonization of leaves; though there may have developed some 'division of labour' between species. "Various types of metabolic interactions both stimulatory and inhibitory between species of fungi and other organisms might also be involved in shaping the observed successional pattern". In other words understanding and explaining successional and seasonal patterns is difficult. There are many plausible explanations; and whilst the gross changes may be easy to understand, the behaviour of many individual species cannot yet be pinned down. Additional information on the physiological and competitive capabilities of these species may help in this, as well as elucidating the role these fungi have in processing leaves.

2.3.5 Dispersal of aquatic Hyphomycetes.

The appearance of aquatic Hyphomycetes - as spores or as colonizing mycelia - at all times of the year in their typical stream habitat, and their widespread geographical occurrence in streams and rivers, suggest that they have efficient means of dispersal in both time and space as well as being particularly well adapted to such habitats in other ways.

'Passive' non-motile organisms such as most saprophytic fungi always have to contend with spatial and temporal heterogeniety - patchiness - in the suitability of their immediate environment. At a particular time in a particular habitat suitable substrates and conditions will not be uniformly available. Similarly in a particular location suitable conditions will not continue ad infinitum.

For instance, by the end of summer the leaf material of the previous autumn has been substantially processed and numbers of aquatic Hyphomycetes are rather low. Yet when autumn comes and the leaf supply is renewed profuse growth and sporulation occur very rapidly. To do this the potential inoculum of aquatic Hyphomycetes must have been maintained over the "leaf gap". Also aquatic Hyphomycetes are sometimes found in temporary pools which dry up for part of the year (eg. Bärlocher et al 1978). They must have some means of surviving until conditions are again favourable.

There is also a particular problem of living in running water, commented on by Bärlocher and Kendrick (1974) "...the peculiar spore forms of the aquatic Hyphomycetes seem to confer selective advantages by their superior adaptation for attachment to solid substrates. The same feature might also promote dispersal upstream (one of the problems faced by all stream dwellers - Hynes 1970) when the spores become attached to animals moving in that direction."

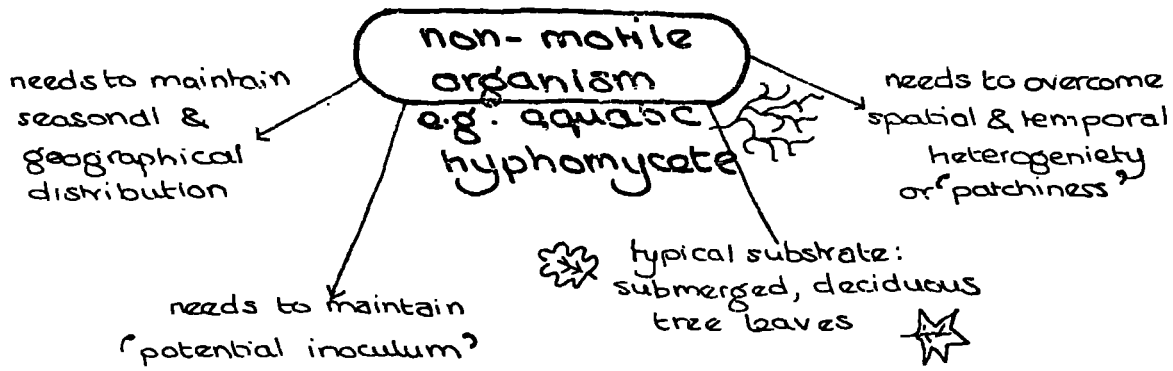
The occurrence of aquatic Hyphomycetes in a variety of habitats, on various substrates, and in different forms stresses their versatility and may help preserve their dominance in their typical stream environment by aiding spatial and temporal dispersal.

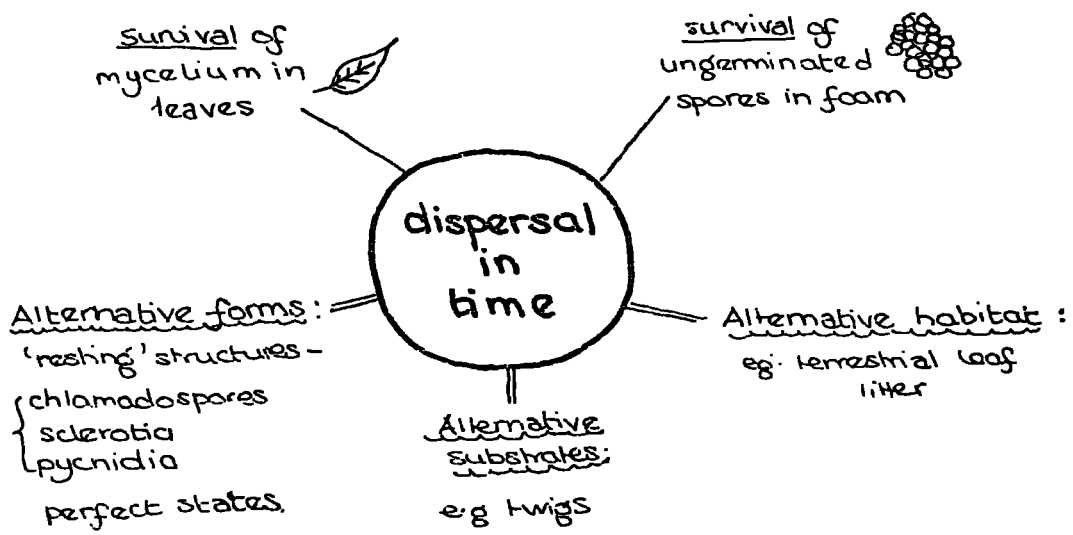
Reasons and strategies for dispersal are set out in Table 2.7. Some of these-alternative forms, substrates and habitats - have been discussed above, as has the survival of ungerminated spores in foam and suspended in water. The particular efficiency with which tetraradiate branched and sigmoid attach themselves to substrates, the problem of 'initial anchorage', has already been touched on.

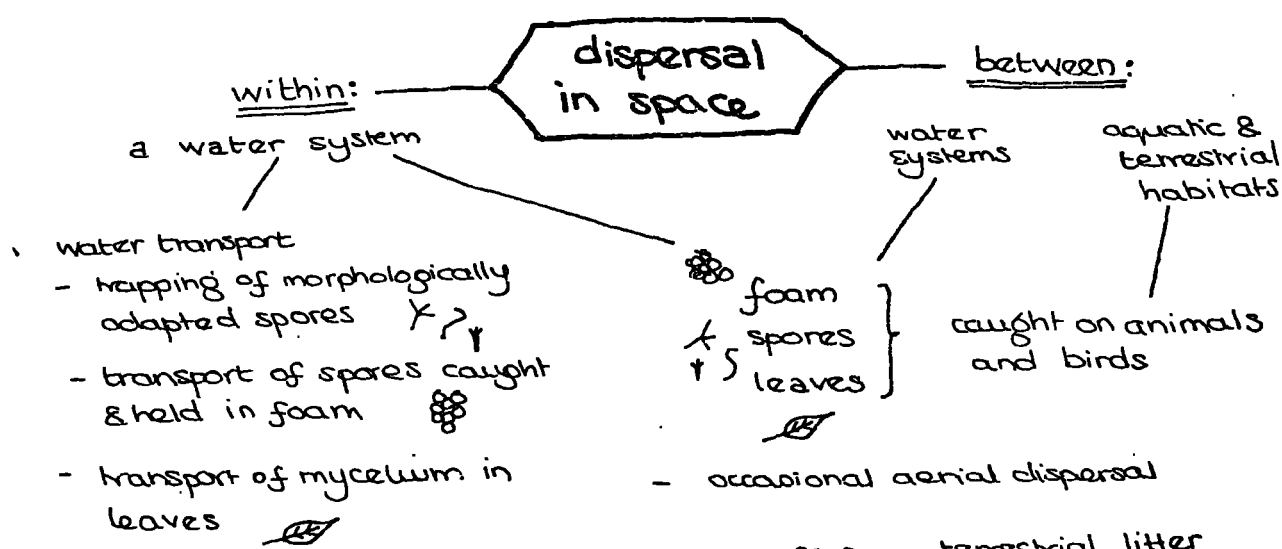
Thakur (1977) in a brief but interesting paper, reports the survival of the mycelia of aquatic Hyphomycetes in dried-out leaves. Submerged

Table 2.4

Dispersal of Aquatic Hyphomycetes







leaves were tested for aquatic Hyphomycetes (and the species found recorded) then dried out and kept for 2 to several months. The leaves were then re-submerged, where they proceeded to sporulate. "The ability of aquatic Hyphomycetes to survive in leaf substrates under dry conditions might explain their recurrence in very isolated seasonal freshwater ponds and streams".

Nilsson provides a comprehensive survey of his own and other peoples observations on dispersal and distribution; the stress is on dispersal in space. Tubaki and Nilsson demonstrated transport of spores in misty spray produced by waterfalls. Slides coated with agar up to 30 metres from the water showed trapped spores after only a few hours. However, Nilsson thinks transport by wind and animals, on dried out leaves and other substrates must be common and more important. He also reports on a rather unusual form of animal transport: the dispersal of V. elodeae, terrestrial fungi and 'snow' fungi from snow patch to snow patch by Lapland reindeers.

2.3.6 Special topics in aquatic Hyphomycete ecology.

2.3.6.1. The terrestrial occurrence of aquatic Hyphomycetes

The terrestrial habitat may influence an adjacent aquatic habitat in several ways. Rain may wash in soil and dissolved salts; leaves from bordering trees and plants may be washed, blown or dropped into the water. The terrestrial habitat harbours many saprophytic fungi, especially in soil and leaf litter. These may be washed in as spores or hyphal fragments or be introduced as colonists on plant material. The importance of terrestrial or geofungi as potential and actual decomposers when introduced into aquatic systems, as well as the occurrence and activity of 'aquatic' fungi in terrestrial habitats are interesting areas of study receiving increasing attention. "It is becoming increasing clear (Bandoni 1972) that many of the fungi generally regarded as aquatic Hyphomycetes also colonize damp

leaves above normal water level and conidia from these leaves may be caught-up in foam when a stream is in spate. Conversely it is also becoming apparent that a number of fungi normally terrestrial can at times grow and sporulate in a perfectly normal manner below water". (Ingold 1974).

Aquatic Hyphomycetes are not rare on land though their role in terrestrial habitats is imperfectly understood. "Since Ingold drew attention to the presence in streams of Hyphomycetes with characteristically complex conidial morphologies, aquatic habitats have been fruitful sources for such fungi so much so that there has been a tendency to refer to such species as 'aquatic Hyphomycetes', irrespective sometimes of their real site of activity" (Park 1974). Most mycologists interested in aquatic Hyphomycetes have, in fact, looked for them, perfectly logically, in their typical habitat.

Bandoni lists the previous handful of terrestrial observations in his 1972 paper "Terrestrial occurrences of some aquatic Hyphomycetes" and also reports his own study. He devised a useful method of observing aquatic Hyphomycetes on various types of litter and found that several species were abundant from the terrestrial habitat he studied, a "high well drained area at some distance from streams or other water bodies".

Pieces of leaf or other plant material were placed in petri dishes and flooded with distilled water. Conidia were lifted into the surface film which was then scanned. Tetraradiate and sigmoid spores were conspicuous amongst the many species of terrestrial saprophytes, and were found to be most common on the leaves from deciduous trees. None were found on leaves taken directly from the tree. Most of the species found were those regarded as versatile or semi-aquatic by Nilsson, for example: Articulospora tetracladia, Tetracladium setigerum, Varicosporum elodeae and Gyoefyella craginiformis.

Laboratory studies on these terrestrial isolates were carried out by Bandoni and in 1974 by Koske and Duncan. Some of their observations on sporulation and temperature have already been discussed, as have similar carried out by Singh and Musa (1977).

In 1974 Park published an interesting experimental and discussion paper "Aquatic Hyphomycetes in non-aquatic habitats" along with another paper describing a Tricladium species, T. terrestre, which he had found "on dead leaves lying on soil in a well drained shrubbery in Chlorine Gardens, Belfast." He used two methods of 'searching' for the fungi: Bandoni's flooding method, and burying glass slides in beakers full of soil. The leaves revealed a number of known species, and T. terrestre. The glass slides bore interesting spores with characteristic morphology, but which could not be isolated. In his discussion Park presents a list of previous 'sitings' giving the species and substrates involved. This includes the classic record of T. setigerum from leaf litter by Scourfield (1940), and the interesting occurrence of V. elodeae on root surfaces (Waid 1954). Park points out that the list "is unlikely to be comprehensive, but is sufficiently extensive to suggest that a consideration of a possible role for such fungi in sites of more or less normal moisture regime may be of interest. The possibility of an active role however, is related to more than just occurrence, or presence of the species at a site, and the records should be examined with this in mind.." He then gives a succinct, lucid account of the difference between the presence and the activity of a fungus, and the interpretations that can be put on observations from various incubation regimes. Rapid induction of sporulation on incubated material suggest that the fungus was already present as hyphae rather than as inactive spore; whilst sporulation "in situ", noted by direct observation of material taken straight from the field, is an even better indication. Thus the

observation of conidia in water films from the submergence of fresh material straight from the ground "is the strongest evidence that sporulation occurred at the soil surface in the natural litter". And he concludes that several of the records thus include observations that indicate activity rather than "mere presence or occurrence". Obviously much more work needs to be done before the contribution of aquatic Hyphomycetes to the decomposition can be assessed. This information would also throw some light on the possible contribution to stream sporas of those aquatic Hyphomycetes washed in from the surrounding watershed. Koske and Duncan think it possible that such a contribution adds to the seasonal abundance of such spores; the terrestrial pattern of occurrence being very similar to that in water, with most spores found in the colder months.

Gareth-Jones in his concluding chapter "Further topics of interest" in "Advances in the ecology of aquatic fungi" (1976) summarizes and discusses this field of study and cites it as an area for further work. Since then several papers have touched on this subject:

Dyko (1976) in a short concise paper reports a preliminary study on the aquatic Hyphomycetes to be found on "partially degraded leaves [which] were selected from woodlots and adjacent streams if available". He confirmed Bandoni's findings that the most productive terrestrial sites were those "which have moderately heavy litter with partially decomposed leaves". Most of the fungi were far more frequent from the stream than in the litter – apart from V. elodeae and T. setigerum. No assessment was made of activity.

In the above reported studies the workers made a point of collecting leaves and other materials from well drained sites. Webster (1977) reports on a year long sampling programme for terrestrially occurring hyphomycetes on an oak wooded slope descending to the River Teign (Devon). In this study

Table 2.8

Aquatic Hyphomycetes recorded from terrestrial habitats up to 1977 (Webster). Includes all species to be found in Ingold's "Guide" (1975a).

	Alatospora acuminata	*
0	Anguillospora longissima	*
0	Articulospora tetracladia	+
	Camposporium pellucidum	+
0	Camposporium species	-
	Campylospora species	-
	Casaresia sphagnorum	*
	Centrospora acerina	+
0	Clavariopsis aquatica	*
0	Clavatospora stellata	*
0	Dendrospora erecta	*
0	Dendrospora species	-
	Flabellospora species	-
0	Flagellospora curvula	*
	Flagellospora species	-
	Gyoerffyella craginiformis	+
	Ingoldiella hamata	+
0	Isthmotricladia species	-
	Lunulospora curvula	*
	Lunulospora species	-
0	Lemmoniera aquatica	*
	Lemmoniera terrestris	+
	Margaritospora aquatica	+
0	Pleuropedium tricladiodes	+
0	Tetrachaetum elegans	*

Table 2.8 (continued)

O	Tetracladium marchalianum	*
	Tetracladium maxilliforme	+
	Tetracladium setigerum	+
O	Tetraploa aristata	+
	Tricellula aquatica	*
	Tricellula species	-
	Tricelophorous monosporus	*
O	Tricladium chaetocladium	
	Tricladium gracile	*
O	Tricladium splendens	*
	Tricladium terrestre	+
	Tripospermum myrti	+
	Triposporium elegans	
O	Triposporium species	-
	Trisulcosporum acerinum	+
	Varicosporium elodeae	+
	Varicosporium species	-
	Volucrispora aurantiaca	+
	Volucrispora graminea	+
Perfect	Nectria lugdunensis (H. lugdunensis)	+
stages	Mollisia species (A. crassa)	

O recorded by Webster (1977) - not necessarily exclusively

\+ associated mainly with terrestrial habitats or regarded as versatile

* associated mainly with aquatic habitats

9 sampling points were pegged out at various levels above the river, starting at the bank (0 metres) and working up to 35 metres. Every month oak leaves were collected from each peg, flooded with distilled water and incubated for a short period. The meniscus, leaf margins and bottom of the petri dishes were scanned for spores and sporophores. Webster made a point of recording "those recognizable spores which had previously been reported from aquatic habitats." Certain trends were apparent; more spores were observed on leaves that had been on the ground for several months; the number of records very close to the river were greater (possibly due to flooding); the frequency of conidia attached to conidiophores was very low and found only within 20m of the river. Those found nearer the river could have been thrown up from the water or deposited in flood, whilst those at the higher levels have an unknown origin. Webster points out that the number of conidia found in the surface film produced from leaves at these higher sites was very low "...this contrasts with the large number of spores which are found, after incubation in water, on leaves removed from streams".

2.3.6.2 Aquatic Hyphomycete Spores

The spores of aquatic hyphomycetes are conspicuous, elegant and distinctive. Because of the constancy of their peculiar morphology many can be identified to species, and very few cannot immediately be placed in the appropriate genus. They are also easily retrieved from streams, lakes and rivers in the foam and scum which collects against barriers such as twigs for example, or against the bank. "Thus spores found in scum samples are to mycologists what plankonicalgae gathered in the plankton net are to algologists. We can study the hyaline, thin-walled radiately branched fungus spores in scum samples directly under the microscope without applying any

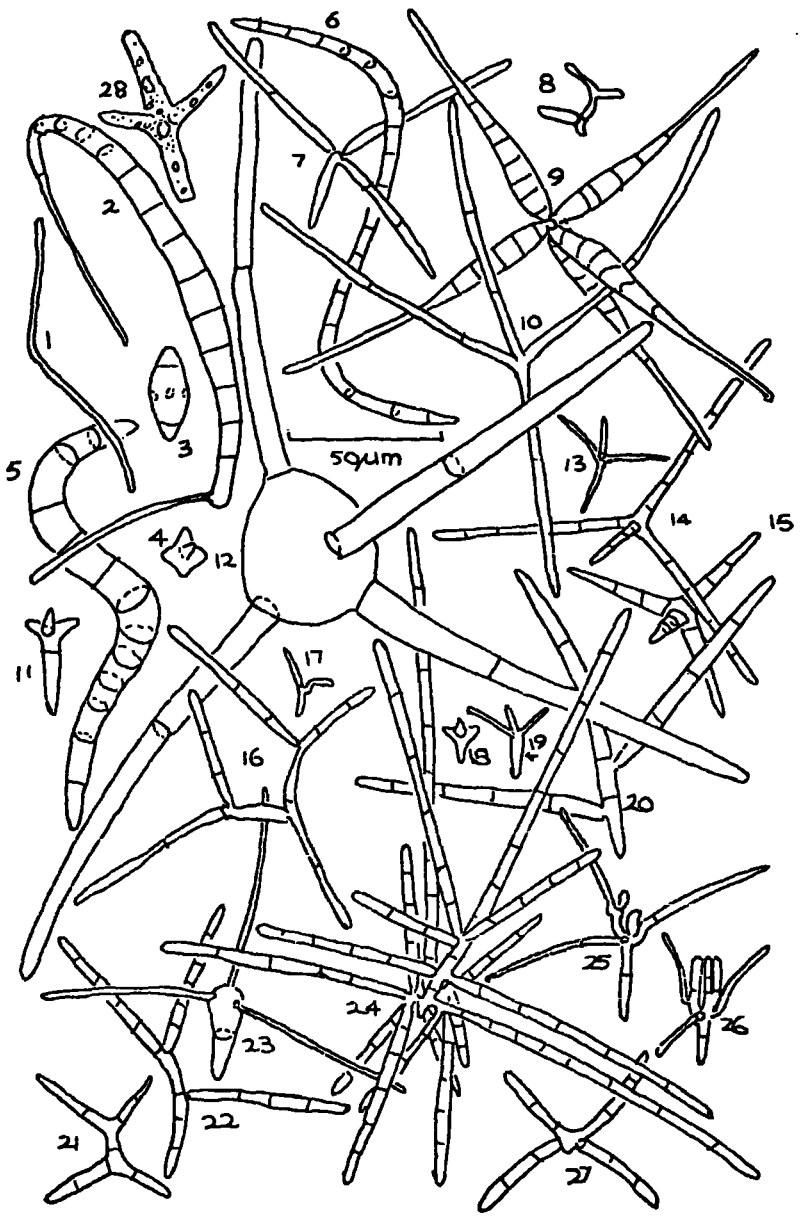
mycological techniques to the sample" (Muira 1974).

Spores can also be trapped, for instance by placing glass slides in streams (mimicking a leaf or twig); or can be collected on a special filter as stream water is drawn through it.

Thus ease of collection and identification has made these spores a useful tool in the compilation of species lists and in the study of aquatic Hyphomycete ecology. However spora may be rather misleading. For instance the spora revealed by a foam sample may not be the same as that produced by filtering at the same site. Sporas compiled by the use of any method may not accurately reflect the aquatic Hyphomycete flora at that site. Qualitative and quantitative discrepancies exist between different 'types' of spora and between sporas and floras. These discrepancies are partly due to the methods used and partly to actual differences between the abundance and variety of inactive spores and active, growing hyphomycetes. Thus the effects of turbulence, and the impact of aquatic Hyphomycete conidia from the land in runoff may affect the spora without substantially altering the flora.

Collection, fixation, staining and microscopical examination of foam, scum and film is an easy and rapid procedure and gives useful qualititative information on the species to be found, if not just at that site, at least in the 'catchment' area. Ingold has used such collections to good ecological and artistic effect. His illustration of a spora compiled from a single foam sample is given as Figure 2.12. Nilsson reported that "in some large streams or stream systems, 75% of the Swedish species may be represented in one sample of foam" (N64). Willoughby and Archer (1973) made extensive use of spore collections in a long term study of fungi in a lakeland stream; they took terrestrial contributions to the spora into account making an interesting comparison between the mainly aquatic Hyphomycete spora found during dry

Fig. 2.12



Spora of Redlake River based on single foam sample. 1, Flagellospora curvula; 2, Centrospora acerina; 3, Dactylella aquatica; 4, Margaritispora aquatica - Ingold; 5, Anguillospora crassa; 6, A. longissima; 7, Articulospora tetracladia - Ingold; 8, Tricladium sp.; 9, Flabellospora sp.1; 10, Tetrachaetum elegans - Ingold; 11, Heliscus lugdunensis; 12, Actinospora megalospora; 13, Alatospora acuminata; 14, Lemonniera aquatica; 15, L. terrestris; 16, Varicosporium elodeae - Kegel; 17, Voluscrispora graminea; 18, Clavatospora stellata; 19, C. longibrachiata (Ingold) Nilsson: 20, Tricladium splendens; 21, T. angulatum; 22, T. patulum Marvanova & Marvan; 23, Clavariopsis aquatica; 24, Dendrospora erecta - Ingold; 25, Tetracladium marchalianum; 26, T. stegerum; 27, Lemonniera cornuta - Ranzoni; 28, rhizopod? Contents shown only in 28.

weather and the more diverse spora of aquatic, aero-aquatic and terrestrial fungi found during wet weather.

Bärlocher et al (1977) immersed glass slides,coated with a thin layer of rosin, in a stream; retrieved them after a few days, stained them and scanned them under a microscope. Conidia of aquatic Hyphomycetes were found adhering to the film; some had started to germinate. Comparison of the species trapped with those retrieved by filtration gave good agreement and Bärlocher suggests that such slides may provide "a simple and fast evaluation of the mycoflora of streams".

Linsey and Glover (1975) used two types of impaction trap to intercept spores of aquatic Hyphomycete. The trapping surface was either flat (a supported sheet of cellophane) or curved (cellophane film wrapped round a metal rod. These traps were inspired by Webster's trapping experiments (1959) and simulated leaves, stems and small twigs . The flat traps caught more spores than the curved(contrary to theoretical considerations), probably due to the sweeping away of spores by passing leaves. They found that the small spore of Alatospora acuminata was trapped more efficiently on the flat surface whilst the much larger Tetrachaetum elegans spore was trapped more efficiently on the rod. Lindsey and Glover compared numbers of spores and species retrieved by foam sampling with those trapped on cellophane: quite large differences emerged—of 29 species recorded 27 were found in foam whilst only 17 were found on traps (in spite of larger number of spores analysed from trap samples). They noted Webster's findings that tetraradiate spores impact more than filiform and concluded that this would lead to the impaction samples being biased with respect to the total spora. They also noted the selectivity of foam (Iqbal and Webster 1973a), tetraradiate spores being more readily removed from suspension than other spore shapes. Comparison of these selective processes suggested that foam would be the more selective of the two:

Their results supported this with the total frequency of filiform spores in foam being 8.1% whilst the frequency on traps was 30.2%. They suggested that "the impaction picture of a stream spora has, possibly, a greater ecological significance than a foam spora, since Webster (1959) has shown that the tetraradiate and filiform spore-types are essentially adaptations for efficient impaction".

Iqbal and Webster (1973a) contributed greatly to the understanding and use of spores in the study of aquatic Hyphomycetes by interesting laboratory experiments on the trapping of spores by air bubbles. Also by a revealing comparison between the qualitative and quantitative composition of spore samples from foam and by filtering. These experiments and observations preceded their important field study of the seasonal variations within and between the spores of two contrasting river systems. In the filtration of water to retrieve the suspended spores a known quantity of water was drawn through millipore filters of pore size 8.0µm. These filters were then stained and rendered semi-transparent so that the spores showed clearly when the entire disc was scanned under a microscope. The spore numbers and species composition that Iqbal and Webster revealed by foam and filter samples are set out in Table 2.9. The comparison between the qualitative foam sample and the quantitative filter sample shows that a larger proportion of filiform spores such as F. curvula and L. curvula were trapped by the filter; a larger proportion of common tetraradiate forms such as T. marchalianum and A. tetraradiate being caught in the foam. In the light of these results, Iqbal and Webster decided to examine the efficiency with which bubbles rising through spore suspensions removed spores of different morphology.

Bubbles of known size were passed through single spore suspensions, and through mixed spore suspensions, to assess the rate of removal of spores and

Table 2.9

Numbers and percentages of spores of aquatic hyphomycete in a 10 ml filtered foam sample and a 1000 ml filtered river water sample from the River Exe,4 September 1970

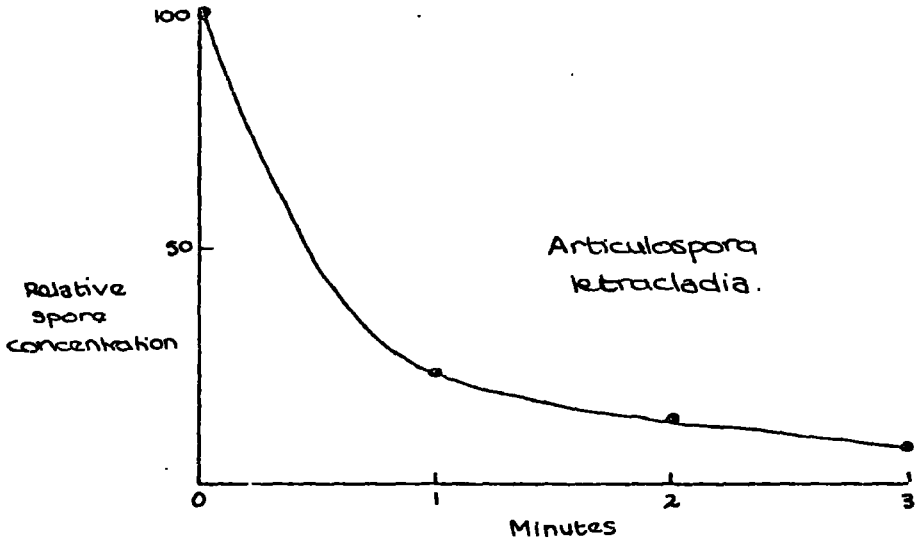
	foam		river water	
Species	Nos.	%	Nos.	%
Anguillospora longissima	2	5.8	75	7.2
Articulospora tetracladia	6	17.6	10	0.9
Clavariopsis aquatica	0	0	30	2.9
Clavatospora longibrachiata	1	2.9	90	8.7
Flagellospora curvula	2	5.9	120	11.6
Heliscus ludunensis	0	0	10	1.0
Lunulospora curvula	2	5.9	640	61.8
Tetrachaetum elegans	4	11.7	5	0.5
Tetracladium marchalianum	17	50.0	40	3.9
Tricladium splendens	0	0	5	0.5
Volucrispora graminea	0	0	10	1.0

compare the effectiveness of removal of the different types of spore shape. Trapping efficiencies were calculated for each of the 20 spore types tested: these included tetraradiate, branched, curved (2-D and 3-D), straight and round spore shapes. Comparing single spore suspensions they found that "Apart from A. crassa and C. acerina the most efficiently trapped spore appears to be the tetraradiate"

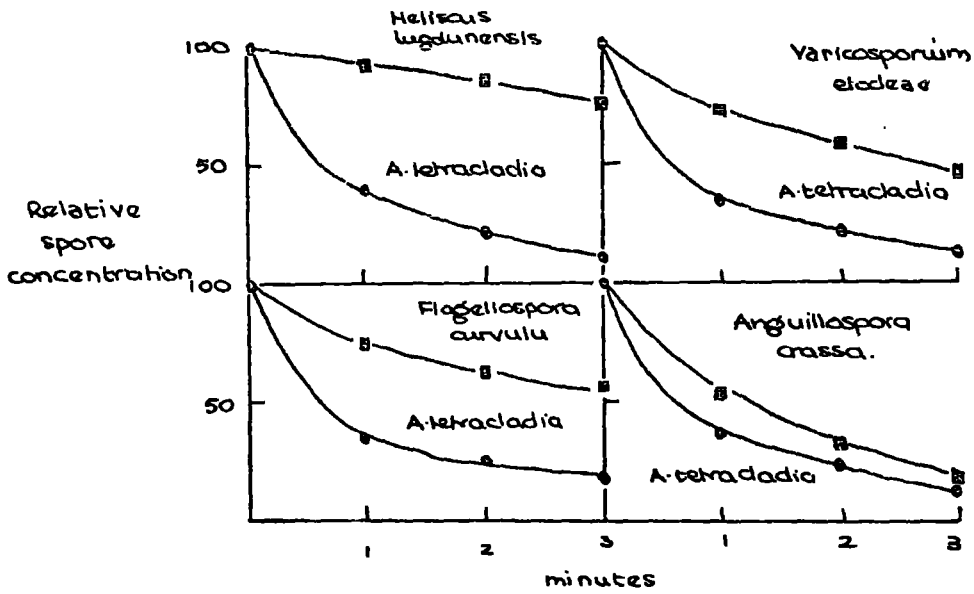
Interesting experiments were done on V. elodeae spores with different numbers of arms. Trapping efficiency increased with the number of arms. Also A. tetracladia f. angulata was found to be less efficiently trapped than f. tetracladia; whilst the aerial spores of H. lugdunensis were less efficiently trapped than the clove-shaped aquaspores.

In mixed suspensions they compared the efficiency of removal of certain tetraradiate forms when paired with other branched filiform and 'conventional' spores. (see Figure 2.13). The results were "broadly in line with expectation"; that is, the tetraradiate spores were more efficiently removed; however C. acerina and A. crassa, were again almost as efficiently removed. The bias in the relative numbers of species in foam is thus explained. Observations on bubbles trapped under cover slips showed that the tetraradiate spores were arrayed with 3 of their arms tangential to the bubble and one arm pointing upwards; whilst sigmoid spores "seem to be able to adapt their curvature to the bubble surface so that the body of the spore is tangential to the bubble surface". They suggest that this explains why bubbles "catch" (and hold onto) spores rather than slipping round them. Bubbles reaching the surface break and fling 'enclosed' spores upwards. Thus a slide held above a bubbling spore suspension will catch the upflung spores, perhaps for observation or isolation.

The development of the filter method, and the direct observation of stained filter discs, thus provides a more accurate and revealing method of sampling aquatic Hyphomycete sporas which is still easily and rapidly carried



Change of spore concentration during 3min period of aeration at 40ml/air/min.



Comparisons of rate of removal of spores from mixed suspensions

(after Iqbal & Webster 1973a) Fig 2·13

out. Filter samples are used to great effect in Iqbal and Webster's study of river spora (1973b) and the excellent resultant line diagrams can be seen in Figure 2.11.

Qualitative foam and impaction methods are still of great use in providing rapid surveys; at least now their limitations are known.

The spora of a stream, river or lake, as mentioned above, does not necessarily accurately reflect the aquatic Hyphomycete flora to be found growing on leaf and other vegetable material in the vicinity. Imported spores may be swept downstream or washed in from terrestrial litter; whilst sporulation may be inhibited or enhanced by a number of physical and chemical factors which do not affect vegetative growth to the same extent. A number of such factors, and the effects they produce, are outlined in Table 2.10. Most of these effects have already been discussed,

Iqbal (1972) noted various discrepancies between the species appearing on submerged leaves and in the river water, as revealed by filtration. For instance, between April and June a number of species found growing on leaves were not detected on the filters. This could be due to the method - filtering probably only shows up spores at certain concentration - but probably also reflects a very low sporulation rate. Also, Iqbal found V. elodeae on leaves from the River Exe but did not record its spores from the river water. At the same time in another river, the spores could be found. Iqbal concluded that this was a pH effect.

The importance of 'imported' spores obviously diminishes in a small stream with no tributaries, though runoff from the land will still occur. Also in a large river system the 'total' spora will more accurately reflect the 'total' flora, at least qualitatively.

Table 2.10

Factors which lead to qualititative* and quantitative+ discrepancies between aquatic Hyphomycete spora and flora.

At a single site			
+ Turbulence:	due to physical obstructions; rapid water flow due to heavy rain	stimulates sporulation	Webster and Towfik 1972
*pH:	may prevent or inhibit sporulation but not growth		Iqbal 1972
*Time of year:	little spore production in summer but growth continues		eg. Iqbal and Webster 1973b
*Introduction of spores from tributary:	increase both the number and variety of spores found		eg. Nilsson N64
*Runoff from water shed:	Introduces spores of terrestrial origin		eg. Koske and Duncan 1974
Between similar sites on the same stream			
+ Number of obstructions in the vicinity			Dyko 1976
*+ Upstream/downstream of tributary			
*+ Amount of runoff from wooded/non-wooded bank			
Differences in leaf input	*qualitative: leaf species involved +quantitative: number of leaves introduced		
*+ Chemical factors, eg. upstream/downstream of sewage input			
Where no flora occurs eg. certain lakes, estuaries			

+* contribution from inflowing streams and rivers. +* runoff from land

Dyko in his 1976 paper "Aquatic Hyphomycetes found in 5 sites in Story County, Iowa" suggests that the difference in the number of spores observed at the various sites was probably due to the number of obstructions present in the stream at each site.

To conclude: foam and scum samples allow rapid surveys of the aquatic Hyphomycete spora, whilst filtration provides a useful quantitative assessment, useful for comparisons in time and (with caution) in space. Neither can safely be used to predict the composition of the aquatic Hyphomycete flora in the immediate vicinity.

And as a final comment: Padgett (1976), in an interesting study of fungal decomposition in a stream in Puerto Rica, makes the following statement: "Since it was suspected that fungi actively engaged in the decomposition of these (leaf) discs were species of freshwater hyphomycetes, it was hypothesized that spore production might serve as an indication of mycelial growth, which in turn, could be correlated with leaf disc weight loss. Accordingly correlation -regression comparison of total spores produced at each harvest and corresponding increments in weight change should have resulted in high correlation coefficients. However the actual computed correlation coefficients were low". And they conclude: "It is important to point out that low statistical correlation of spores to weight loss does not preclude the significance of freshwater Hyphomycetes in decomposition,but merely illustrates that spore production is not an adequate index of mycelial growth".

3. THE ROLE OF AQUATIC HYPHOMYCETES IN DECOMPOSITION

3.1. Introduction

3.2. Decomposition in Aquatic Ecosystems

3.2.1. What is decomposition?

3.2.2. The importance of allocthonous material in aquatic ecosystems

3.2.3. Leaf processing

3.3. Two Major Studies on the Mycoflora of Autumn-shed Leaves

3.3.1. Introduction

3.3.2. Bärlocher and Kendrick's study

3.3.3. Suberkropp and Klugs' study

3.4. Hyphomycetes as Intermediaries of Energy Flow in Streams

3.4.1. Introduction

3.4.2. 'Conditioning' of leaf material by aquatic Hyphomycetes

3.4.3. Consumption, assimilation and preference experiments.

3. THE ROLE OF AQUATIC HYPHOMYCETES IN DECOMPOSITION

3.1 Introduction

Chapter 2 deals mainly with the biology and autecology of aquatic Hyphomycetes. In this chapter their role as decomposers of plant material is highlighted, as is their importance as intermediates in further decomposition by invertebrate detritivores.

Decomposition is a fascinating, vital and universal process, one of the main cogs in the functioning of ecosystems. The first section of this chapter describes the process and progress of decomposition; the importance of decomposition in aquatic systems - especially the importance of allocthonous or 'imported' material to so-called 'heterotrophic' streams; and the way in which autumn-shed leaves - the major part of this allocthonous input - decompose and are processed in streams.

The second section reviews two interesting papers which examine the fungal and bacterial flora of leaves, principally aquatic Hyphomycetes. The successional activities of the leaf microflora are related to leaf decomposition and processing. Both papers stress the difficulty of studying the ecology of micro-organisms and employ well-thought-out comparative methods to get as good a picture of what numbers and species of fungi and bacteria are active in leaf decay.

The last section deals with the contribution that leaf colonizing micro-organisms make to the value of such leaf material as food to stream invertebrates. Autumn-shed leaves are an important input to streams; aquatic Hyphomycetes are dominant colonizers and decomposers of such material; whilst invertebrate detritivores are known to be important in the physical and chemical breakdown of plant detritus in streams. Inevitably workers began to investigate whether these two groups of decomposers interact in the more efficient breakdown of the terrestrial

leaves so important to the energy and materials budgets of the streams in which both groups of organisms thrive.

In drawing together the threads of so many different studies numerous generalisations have been made and borrowed. Reviews and major studies in the areas considered usually contain long lists of important background and historical references. To retain clarity these references have largely been omitted from this account. Interesting and important reviews are referred to and these contain adequate bibliographies.

3.2 Decomposition in Aquatic Ecosystems

3.2.1 What is decomposition?

Mason (1977) gives an excellent account of decomposition in the recent Institute of Biology study of the same name. This includes a discussion on litter production and composition, outlines the process of decomposition including the roles of the microflora and fauna, and gives an indication of the contribution that decomposition makes to the energy flow and nutrient cycles involved in the functioning of ecosystems. The stress is on terrestrial systems - more work has been done on these - but some pertinent information on freshwater habitats is also included.

The Oxford English Dictionary defines 'to decompose' as: "to separate or resolve into its constituent parts or elements". Mason provides an excellent biological definition of the decomposition of a dead plant or animal: "Dead organisms are broken down into large particles, then into small particles and eventually into small molecules. The dead organism is thus gradually disintegrated until its structure can no longer be recognised and complex organic molecules are broken down into carbon dioxide, water and mineral components".

Decomposition involves both physical and biological agents. Decomposing organisms release, use and respire the energy contained in dead material. They also aid the physical processes of weathering and leaching, in breaking down and releasing nutrients so that these may be used again by growing plants. Thus decomposition is an important valve in the two basic flows in ecosystems: the one-way flow of energy and the cyclical flow of nutrients (see Figure 3.1).

Litter, or detritus are collective names given to organic matter in the process of decomposition, often found in "drifts" in both terrestrial and aquatic habitats. Darnell (1967) defines detritus as "all types of biogenic material in various stages of decomposition

which represent potential energy sources for consumer species"; that is, all sorts of dead and discarded plant and animal remains. The leaves, roots, twigs, flowers bud scales and fruits of deciduous trees, for instance, form very noticable accumulations of plant material can be found near the banks of some rivers, lakes and streams. Fine, unrecognisable, broken down detrital material forms an important component of terrestrial soils and aquatic muds and silts. The bodies, faeces and various exuviae of animals form an important but smaller contribution to such litter and detritus.

Such remains contain diverse inorganic and organic molecules which in life contributed to the function and structure of the organisms. Some of these constituents such as sugars, amino acids and soluble salts of potassium and sodium are rapidly released as autolysis, leaching initial weathering and rapid colonization by micro-organisms occurs. Most litter is composed of plant material. Structural components such as cellulose, hemi-cellulose and lignins not only form the bulk of this material but are broken down rather slowly. Lignin is a particularly uninviting and intractable substance to most decomposing organisms.

Mason divides decomposition into three basic processes: leaching, weathering and biological action. These processes occur concurrently, in both aquatic and terrestrial decomposition.

Leaching, by rain in leaf litter or by water flow in streams leads to a rapid loss of soluble substances which may then flocculate to form fine particles, or may be absorbed by aquatic Hyphomycetes, for instance (see Figure 3.4 and 3.5). Leaching also occurs as micro-organisms, animals and abrasion disrupt the material. Weathering is mechanical breakdown due to physical agents such as wind or waves. Further mechanical breakdown can occur as animals move through, sort, eat and defacate material. A simple summary of these processes and the agents

involved is set out in Figure 3.2

A variety of organisms consume and breakdown detritus. Fungi and bacteria are particularly important. The actual species found and the balance between the fungal and bacterial contribution may vary from habitat to habitat. For instance, under anaerobic conditions bacteria are the main agents of decomposition. Different types of plant and animal material may have a different spectrum of fungal and bacterial species and the species may change with the progress of decomposition. Taken as a group such saprophytic micro-organisms are ubiquitous. They may exploit dead material in three main ways: "They may directly decompose the substrate using the energy obtained for growth. They may parasitize other organisms, or utilize their waste products, or they may use the substrate merely as an attachment site, while obtaining their nutritional requirements from the surroundings". (Mason 1977).

Fungi are particularly important in the initial stages of decomposition. Harley (1971) in his fascinating paper "Fungi in Ecosystems" gives a succinct summary of the particular features of fungi which make them effective as primary invaders of dead material. "Although fungi, like other heterotrophs, present to their substrate a very large surface, compared with their volume, their hyphal structure endows them with the additional property of spreading and penetrating... The combination of hyphal structure with the production of external enzymes allows them to penetrate deep into the matrix on which they develop. This is especially important in the exploitation of plant material where the cell contents, which may contain certain essential nutrients, are enclosed in resistant walls. It is because of their possession of hyphae that fungi are especially important in the breakdown of leaf litter, of wood and as plant pathogens. By contrast bacteria tend to be restricted to surfaces and to be more important

figs 3·1 & 3·2 after
Mason 1977

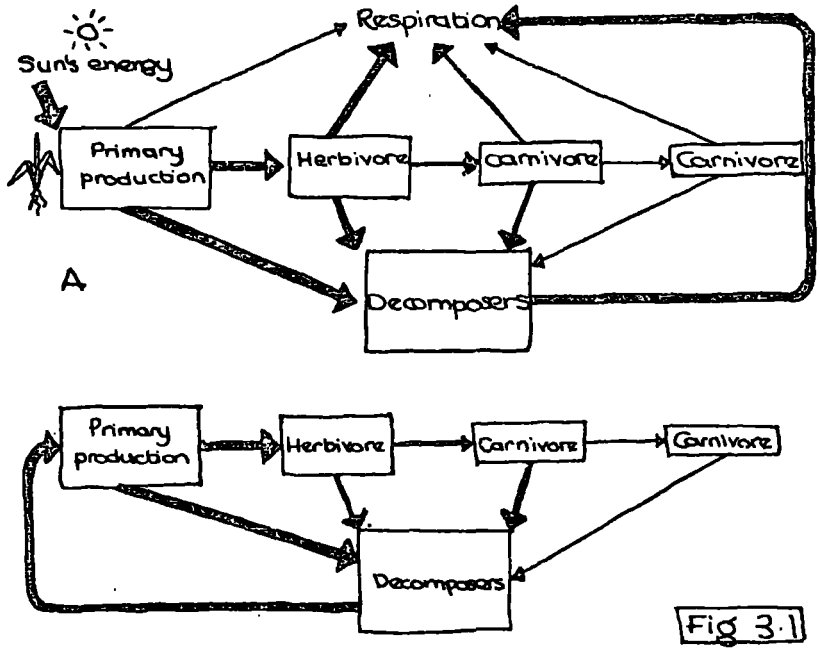


Fig 3·1

A The flow of energy: the boxes represent standing crops or biomass and the arrows fluxes

B The cycling of nutrients: the boxes represent nutrient pools and the arrows fluxes.

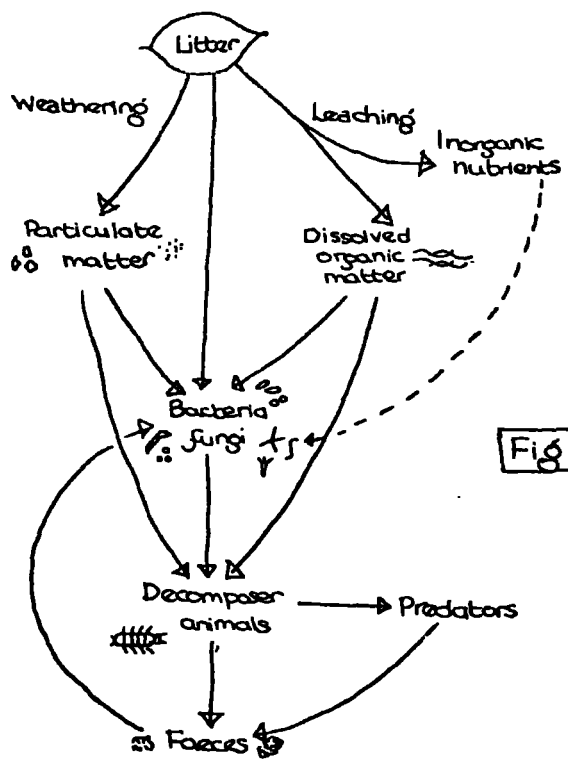


Fig 3·2

Summary of the breakdown process

where material has been mechanically disrupted and its available surface increased". The action of fungi and animals thus increase the suitability of material to bacteria.

All biological molecules, both simple and complex can be exploited by at least some species of fungi. Most species can use simple compounds such as sugars as an energy source though the rapid exploitation of such compounds in the field is usually due to the presence of certain vigorous, often ephemeral species known collectively as "sugar fungi". These "rapidly exploit transient substances" (Harley 1971). More refractory substances such as cellulose and lignin are characteristically utilized by potentially much longer lived fungi in the ascomycetes, basidiomycetes and fungi imperfecti.

Aquatic Hyphomycetes appear to be particularly versatile. Physiological studies (see 2.2) have shown that they are capable of using a wide variety of simple carbon compounds as energy sources. The suggestion is that most can also breakdown cellulose. On land competition from fungi such as the 'sugar fungi' restricts the usually slower growing cellulose decomposers to their specialist substrate. However in a stream after leaf fall the cold temperature inhibits the growth of such fungi whilst the aquatic Hyphomycetes with their lower temperature optima for growth and sporulation, rapidly colonize the leaves and dominate the mycoflora.

The suitability of a substrate for colonization by particular species or groups of organisms will change with the continuous change in its physical and chemical state. Thus exploitation by bacteria increases as the substrate is fragmented, and colonization of leaf material by

cellulytic fungi increases as the more easily utilized carbon sources are depleted. Successions of this kind in the fungi colonizing terresterial leaf material are well documented by Hudson in his interesting paper "The ecology of fungi plant remains above the soil"(1968).

Successional patterns are largely influenced by the chemical and physical condition of the substrate but may also be affected by environmental conditions.

Thus cold stream temperatures effectively cut out the first stage of succession observed on land in the autumn-shed leaves mentioned above. Less is known about successional patterns in aquatic systems than on land. Interesting observations by Bärlocher and Kendrick (1974) and Suberkropp and Klug (1976) are discussed in 3.3

Mason states that "in terms of weight loss of material, the microflora are probably responsible for about 90% of decomposition in both terrestrial and aquatic habitats".

This is a combination of 'primary' decomposition before the material is consumed by detritivores, and subsequent colonization and breakdown of material eaten but not assimilated by these animals. Aquatic Hyphomycetes are particularly important in this first stage before the material enters the microflora/fauna/microflora cycle illustrated in Figure 3.2.

The fauna play an essential part in decomposition, however. Experiments have been carried out in both aquatic and terrestrial habitats, where detritivores are excluded from decomposing plant material by the use of fine mesh. The material does eventually decay but it takes much longer to do so, remaining recognizable for much of this time. Thus turnover of trapped nutrients and exploitation of energy is delayed. In a forest litter layer the fauna help in the physical breakdown and incorporation of the leaf material into the humus and soil layers; a similar

process occurs in aquatic systems.

A great range of animals is involved in the breakdown of plant and animal detritus. Larger animals such as scavenging gulls and detritus eating fish contribute. But more important are the micro and macro invertebrates which live amongst and feed on this material. Some, such as protozoans and nematodes, are ubiquitous whilst others are characteristic of land, freshwater or the sea. "In freshwater habitats, tubificid worms, chironomid (diptera) larvae , isopods and amphipods are important". (Mason 1977) As in colonization by micro-organisms, the type of material involved as well as the stage of chemical and physical breakdown it has reached will affect the species found "successions of fauna have been observed caused by changes in climate, the suitability of food and colonizing ability" (Mason 1977). Although detritivores "are often considered to be unspecialised feeders... subtle differences in diet can be detected. Thus age of the litter, the species of litter and its spatial position will affect its palatability". Thus in an investigation into decomposition of autumn-shed leaves in streams Kaushik and Hynes (1971) showed that a small number of invertebrate species had a particular order of preference for different species of leaf - rapidly decomposing leaves being preferred to those which decomposed more slowly. (See 3.2.3) They also made a very important observation on the invertebrates preference for leaves with a colonizing microflora, as opposed to those without. The importance of the microflora to the invertebrates is indicated by the following observation from Berrie's 1976 review paper " Detritus, micro-organisms and animals in freshwater":

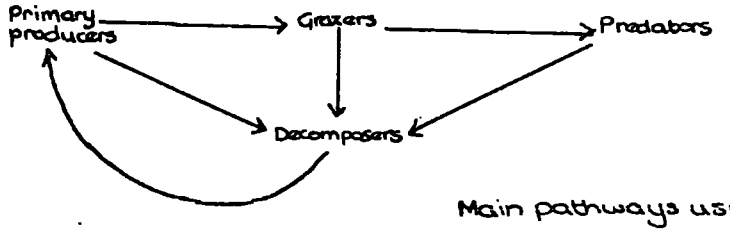
"Detritus is a major source of food for micro-organisms and animals in fresh water ecosystems. It appears to be a low quality food for animals. They pass it rapidly through their guts and are only able

to assimilate a small proportion of the material ingested. There is evidence that they utilize the micro-organisms attatched to the detritus rather than the detritus itself and these are a high quality food. The micro-organisms are also important in making the detritus attractive to animals and less refractory to digestion".

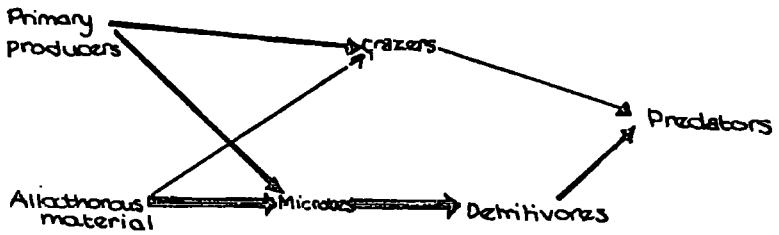
Aquatic Hyphomycetes have their own part to play in this 'conditioning' of plant material for invertebrates in streams (see 3.4).

Invertebrates 'specialize' in the way they consume detrital material: most stream invertebrates involved in leaf breakdown in streams can be described as 'shredders', 'scrapers' or 'collectors'. (see Figure 3.4). Shredders bite chunks out of leaves, or large pieces of leaves, consuming micro-organisms along with the leaf. They are largely responsible for the skeletonization of leaves and are particularly important in the first stages of leaf processing (see Figure 3.4). Scrapers scrape away at the surface of leaves, consuming the encrusting hyphae, bacteria and any algae as well as leaf material. Collectors sort through and consume the fine detrital particles which have entered the second and subsequent stages of processing (see Figure 3.5).

Thus efficient processing of detrital material depends on a combination of physical and chemical processes, especially important being the combined activities of the microflora (with its invasive fungal colonization, surface bacterial colonization and wide spectrum of enzymic ability) and the fauna, which although it cannot contribute greatly to the chemical breakdown of much detrital material, accelerates the process of decomposition by fragmentation.



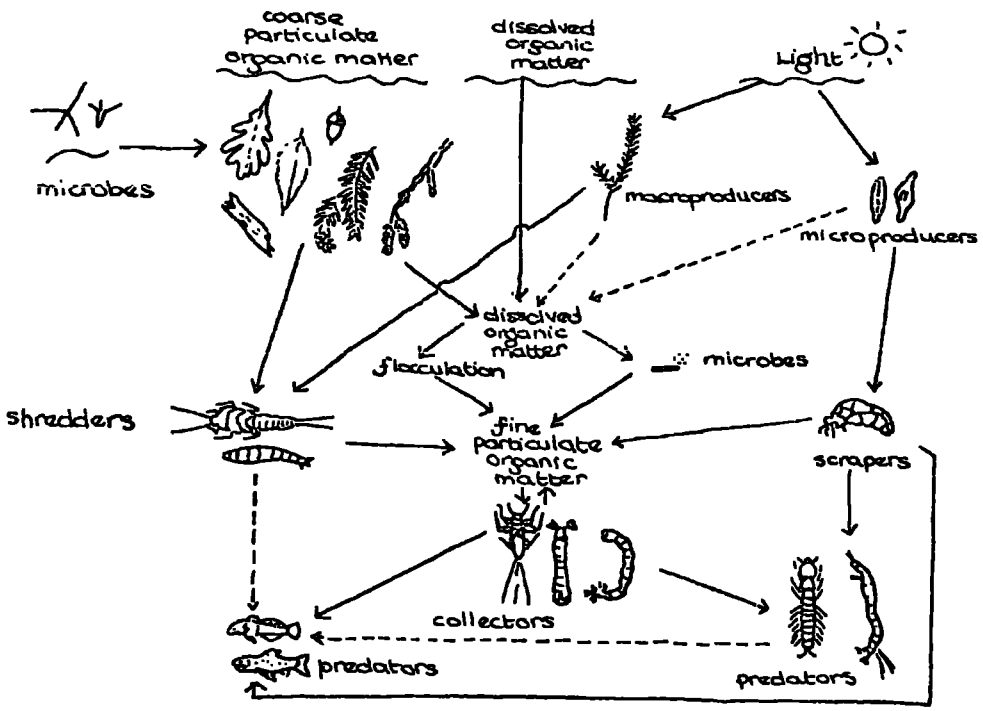
Main pathways usually described for the transfer of materials in ecosystems



Main trophic pathways in freshwater ecosystems.

after Berrie 1976

Fig. 3.3



A conceptual model of stream ecosystem structure and function (modified from Cummins 1973) emphasizing the processing of particulate and dissolved organic matter.

CPOM - exemplified as deciduous leaves, coniferous needles, a twig a nut, bark, and flowers.
Initial colonization of CPOM - represented by aquatic hyphomycete spores.
Utilization of DOM - rod shaped & spheroid bacteria
Macroproducers - e.g moss Microproducers - e.g diatoms
Shredders - e.g stonefly nymph, cranefly larvae
Collectors - e.g blackfly and midge larvae and mayfly nymph.
Scrapers - e.g caddisfly larva predators e.g fishfly and midge larvae and fish.

Fig 3.4

3.2.2 The importance of allocthonous material in aquatic decomposition.

In aquatic systems such as lakes, ponds, rivers and streams there are two sources of detrital material: 'autochthonous' or indigenous material such as dead aquatic plants produced within the habitat; 'allocthonous' or imported material which may be dropped, swept or washed in from adjacent terrestrial and aquatic habitats. Streams may wash large material, such as leaves, as well as small particles into lakes and rivers. Rain may wash in dissolved organic material and inorganic salts from the soil,; some of this may flocculate to form fine particles. Rain and wind may deposit the leaves, flowers and twigs of terrestrial plants in the water.

The relative contributions made by autochthonous and allocthonous material depend very much on the bank: area ratio. "Allocthonous litter is especially important where the edge of the aquatic habitat is large relative to its surface area, notably in rivers, streams and ponds". The factors which affect the balance of allocthonous and autochthnous material in the detrital 'pool' are also important when the relative contributions of detrital breakdown and primary production by plants to the energy budget of the ecosystem are considered.

In a small forest stream the bank: area ratio is large and a great deal of leaf material is shed into the water. Because the stream is shaded by the trees which produce these leaves little light reaches the water. This shading, as well as the scouring action of the stream means that primary production is very low and often restricted to diatoms and other algae firmly attached to stones. Even in rivers where macrophyres thrive studies indicate that the abundant species of benthic invertebrates prefer detrital material to fresh green plants (eg. Scorgie 1976). The macrophytes seem to be more important as sites of attachment and shelter. Thus streams and rivers flowing through forests have communities almost entirely supported by the input of materials from the land and this heterotrophic nutrition is typical of most

running waters which have not been altered by man (Mason 1977).

3.2.3 Leaf processing in aquatic ecosystems

The importance of allocthonous material to woodland stream ecosystems, especially the importance of autumn-shed leaves to temperate streams, has led to many recent studies which examine different aspects of their decomposition in water.

The course and rate of decomposition in several species of leaf has been followed and measured in many ways* and related to (amongst other things) the activity of colonizing fungi and invertebrate detritivores. Other studies have attempted to assess the contribution to the energy budget and nutrient pool of streams and rivers by measuring leaf input and leaf resources (eg. Krumholtz 1972). Other people have tried to co-ordinate the information from various sources and build up a picture of the functioning of a stream ecosystem (Cummins 1973 and Figure 3.4).

For example,

Suberkropp et al (1976) have produced an excellent paper "Changes in the chemical composition of leaves during processing in a woodland stream", which follows the changes in the concentration of major molecules, such as cellulose and lignin, in two species of leaf whoe microflora they also study (1976a). In Petersen and Cummin's paper "Leaf processing in a woodland stream" (1974), daily weight losses in leaves were monitored as leaching, micro-organisms and invertebrates contributed to their chemical breakdown and fragmentation (Figure 3.5).

Several papers deal with the microfloras and invertebrates involved. Studies on fungi have used increase in protein content of the leaf, microbial respiration, and fungal biomas (length of hyphae per unit leaf area) as indicators of fungal colonization as decomposition

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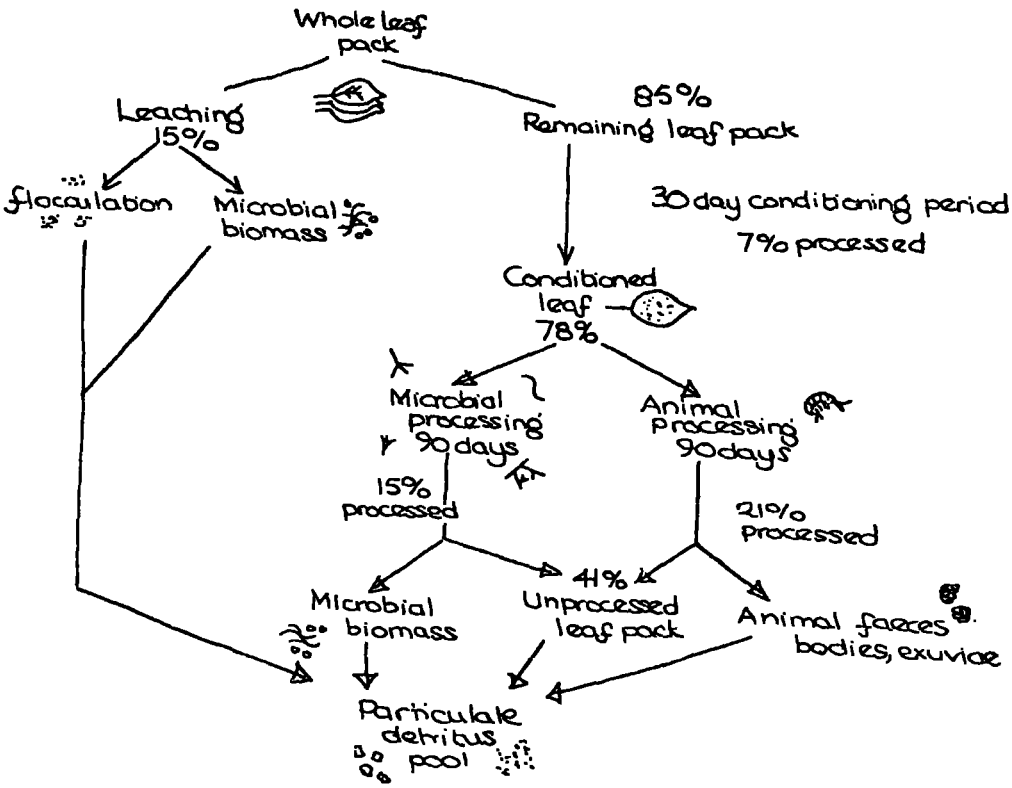
* Suberkropp et al (1976) give a brief but detailed review in the introduction to their paper.

progressed. Whilst loss of leaf area has been used as an indication of shredder activity.

Since aquatic Hyphomycetes form a dominant flora on autumn-shed leaves, attention has been focussed on their particular contribution to leaf breakdown. These fungi are particularly associated with the first stage of leaf processing. This is the stage of processing most studies deal with, whilst the leaf is still conveniently recognizable. It occurs between the initial leaching of the newly submerged leaf and the disappearance of the recognizable leaf as its fragments enter the next stage of processing. This is illustrated in Figure 3.5, Petersen and Cummin's processing budget for leaf packs (experimental analogues of natural leaf accumulations). Here the authors have used data, from their own and other peoples investigations, to build up a picture of the contributions made to leaf processing by the various agents of decay.

In this budget the microflora, principally aquatic Hyphomycetes in a tree bordered stream, contribute in two main ways to this first stage of processing. 'Conditioning' of the leaf, before it becomes attractive to and is consumed by invertebrate detritivores, is due mainly to fungal colonization: initial breakdown of the leaf by fungal enzymes and conversion to fungal biomass. During joint microbial/invertebrate processing the aquatic Hyphomycetes will continue to be the principal fungal colonizers. Petersen and Cummins calculated the microbial contribution to joint processing by respiratory measurements (the leaf is dead and any respiration is due to its microflora). However, the invertebrates are constantly removing leaf and attached fungal material; and this measure of fungal activity may represent a steady state between increasing fungal biomass and removal by invertebrates. The actual contribution to this stage of processing by the fungi may therefore be greater than the budget figures for conditioning

Fig. 3.5



Summary processing budget for leaf material after Peterson & Cummins 1974

Data mainly from studies of hickory (Carya glabra) since this is processed neither very rapidly nor very slowly.

On the 85% of the leaf pack left after leaching there is an initial period of microbial colonization or conditioning. This is followed by animal-microbial processing. The remaining 41% of the leaf pack can continue to be processed by microbial and invertebrate action. However in terms of the actual time scale, after 120 days (Sept. to Dec.) leaf packs probably are rare in natural woodland streams. The material has become fragmented and has entered a different (smaller) particulate detritus category subject to a different ecology.

and joint processing suggest (see Figure 3.5 and section 3.4)

A time scale is also given; this is for hickory, a"species [which] falls in the middle of the continuum of processing coefficients". That is, it decomposes neither particularly rapidly, nor particularly slowly with respect to other leaf species. Variations occur in the rate of processing between species, and to a certain extent within species where leaves may have developed under different growth conditions (shade leaves and 'sun' leaves, for instance).

Differences may be due to morphology: leathery, tough leaves decompose more slowly, for instance. Differences in chemical composition may also affect processing rate: some species have a higher proportion of lignin than others; whilst the presence and amount of polyphenols is particularly important. Tannins in oak and beech leaves, two species which decompose more slowly, are a good example. Not only do these substances precipitate proteins, making them less available to consumers; they may also interfere with the function of digestive enzymes. Fast leaves such as elm, rapidly become soft and unrecognizable as they are quickly exploited. Slow leaves such as beech may persist into the second year of processing as brittle, whole leaves or as skeletons. Intermediate species such as willow, are not as rapidly exploited as the fast species but are unlikely to persist as recognizable leaves through the summer after leaf fall. Streams which receive the leaves of several species of tree, of differing rates of decay will thus supply a series of substrates to their microbial and invertebrate decomposers as the leaves become acceptable to these organisms. Bärlocher and Kendrick (1976) assume this is a natural rationing process (see 3.4).

3.3 Two Major studies on the mycoflora of autumn-shed leaves

3.3.1 Introduction

This section examines the approach, methods, results and conclusions of two interesting and important papers. Bärlocher and Kendrick in their 1974 study: "The dynamics of the fungal population on leaves in a stream" combine long-term field experiments with parallel laboratory studies to build up a picture of fungal colonization and succession on leaves before and after they become submerged in streams.

Bärlocher and Kendricks' results, comments and conclusions are taken into account in Suberkropp and Klug's later paper "The fungi and bacteria associated with leaves during processing in a woodland stream". This is an excellent study, careful and comprehensive, investigating the seasonal and successional occurrence of the dominant species of aquatic Hyphomycetes and comparing their activity with the bacteria and other fungi revealed by the various incubation and observation techniques they employ.

These studies not only increases our understanding of the role of aquatic Hyphomycetes in leaf processing, and the relative importance of aquatic fungi, terrestrial fungi and bacteria. They also make a valuable contribution to the study of fungal ecology. This is particularly true of Suberkropp and Klugs' paper. In both studies well thought out comparative methods of revealing the leaf microflora are skilfully employed. The choice, use and discussion of these techniques is an important part of both papers: and they are careful to point out the influence of their chosen techniques on the results gained and conclusions drawn.

Both studies result from an interest in the importance of

allocthonous organic matter to woodland streams and in the role of micro-organisms, especially fungi in leaf processing.

"Only a small fraction of the energy represented by the leaf material can be directly exploited by animals... for access to the remaining energy the animal community depends on the intervention of micro-organisms, with their greatly superior ability to degrade such plant substances as cellulose and lignin which they exploit in increasing their own biomass" (B&K)*. And from S & K's* introduction: "It has been concluded that at least during the early stages of leaf processing in streams fungi are more dominant members of the microflora than bacteria. One group of fungi, the aquatic Hyphomycetes has been repeatedly observed on decomposing leaf litter in streams on a world-wide basis. Ecological studies have suggested a major role for these fungi in the processing of leaf material".

B & K had already studied the influence of various fungi, including aquatic Hyphomycetes, in increasing the palatability and value of leaves as food for stream invertebrates (see 3.4). In this study they wished to find out more about the populations of leaf decomposing fungi themselves.

S & K's study is part of a continuing programme "to examine the interactions among micro-organisms, organic matter and invertebrates involved in the processing of leaf litter". This paper, on the occurrence and activity of the leaf microflora, was accompanied by an equally well-thought out study on the "Changes in the chemical composition of leaves during processing in a woodland stream". (Suberkropp et al 1976). This allowed the authors to relate changes in the microflora with changes in the condition of the leaf.

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* Since these authors will be frequently referred to I have abbreviated their names to B & K and S & K.

3.3.2 Bärlocher and Kendrick's study

In their main field programme B & K introduced autumn-shed leaves into a stream,retrieving samples at intervals to assess the progress of leaf colonization and decay.

Some of this leaf material was incubation in various ways to allow the fungal population to be isolated and identified.

In order to measure the overall progress of leaf breakdown, loss of weight of leaf material was measured. The biomass of fungi colonizing the leaf was indicated by measuring hyphal length per unit area, whilst the activity of invertebrate shredders was followed by measuring loss of area as the leaves became skeletonized.

This main field programme was augmented by investigations into the microflora of leaves taken straight from the tree and from litter. This indicated which fungal species the leaves might take with them when they entered the stream.

In a parallel series of laboratory experiments selected fungi, found to be important in the observed mycoflora of the leaves, were inoculated onto sterilized leaf discs so that their activity under a variety of conditions could be assessed.

B & K's aim was to record the active mycoflora from their introduced and retrieved leaves. Such an undertaking is not without its difficulties and confusions (see 3.3. & 4.1). In order to identify the microfungi colonizing leaf material, the material may have to be incubated to induce sporulation. Incubation may not only induce sporulation and growth in the fungi active on the leaf, however. The spores of fungi present on the surface of the leaf may also germinate, grow and sporulate. Fungi which have colonized the material but which are inactive because environmental conditions are unfavourable may also be stimulated to vigorous growth and sporulation by laboratory

incubation conditions.

Bärlocher and Kendrick realised that aquatic Hyphomycretes were likely to be important active members of the mycroflora and they wanted to make sure that the methods they used would allow these rather slow growing fungi to show themselves. Preliminary experiments to find suitable media and incubation conditions were carried out. Isolation of hyphal fragments from leaves, whilst ensuring that only 'active' fungi are placed off, they found 'tedious and unsuccessful'. The common mycological procedure of dilution plating (homogenizing the fungus bearing material and incorporating into the incubation media) had already been shown to be unsuitable. They therefore decided to plate small pieces of leaf directly onto a range of media. Malt extract agar is a widely used medium for fungal growth and isolation. It supports the growth of aquatic Hyphomycetes in isolation.

Any other fungi present on the leaf would also grow on such a medium. The other two media used were plain water agar and leaf agar (water agar with a very small amount of homogenized leaf added). Such agars are capable of encouraging quite adequate growth of aquatic Hyphomycetes whilst the growth of 'weed' fungi (not necessarily active on the leaf but very active on conventional laboratory media) is restrained. These plates were incubated at room temperature (near the optimum for many fungi) and at the prevailing stream temperature (much more favourable to the cold adapted aquatic Hyphomycetes).

Such a range and combination of media is likely to reveal much more about the active and inactive (but present) mycoflora than the use of one set of incubation conditions

The main field experiments were planned so that B & K could study the effects of the following influences on the mycoflora they recorded:

The influence of the substrate: by the use of three species of leaf, and the examination of lamina and petiole separately.

The influence of the sampling date: presumably due to a combination of environmental influences and the condition of the leaf.

The influence of terrestrial fungi: phylloplane fungi are present (mainly as spores) on leaves from the tree and litter fungi may colonize fallen leaves, before these drop or fall into the water. B & K sterilized one set of leaves before immersion, to eliminate these fungi.

The influence of incubation conditions: this has been discussed above (and see 3.3.3) B & K were particularly interested in the difference between aquatic Hyphomycete records from the different media.

The following table shows the various combinations of substrate and incubation conditions used during the two field experiments.

TABLE 3.1

AMPLES	LEAF	LEAF PART		MEDIA WATER ONLY	TEMPERATURE
IRST XPERIMENT					
samples an - Aug 1972	Maple	Lamina:	Pre-sterilized not sterilized	Malt Agar	Room Temperature
		Stalk:	not sterilized	Leaf Agar Water Agar	Stream Temperature $0^{o}C$ $5^{o}C$ $10^{o}C$ $15^{o}C$
COND XPERIMENT					
samples t - Jan 72 1973	Oak Maple Ash	Lamina only		Water Agar	$10^{o}C$

TABLE 3.2

1. TERRESTRIAL FUNGI

(a) Mucorales

1. *Mucor* sp. - uncommon
2. *Rhizopus* sp. - uncommon

(b) Hyphomycetes

1. *Alternaria* - mostly *A. alternata* (Fr.) Keissl., and some *A. longipes* (Ell. et Ev.) Mason - very common on senescent leaves.
2. *Aspergillus* - *A. clavatus* Desmazières, *A. flavus* Link, and *A. niger* v. Teighem - all fairly common in spring and summer.
3. *Aureobasidium pullulans* (De Bary) Arnaud - very common on senescent leaves.
4. *Botryotrichum piluliferum* Sacc. et March. - uncommon.
5. *Botrytis cinerea* Pers. ex Pers. - fairly common on senescent leaves.
6. *Centrospora acerina* (Hartig) Newhall - very common in summer.
7. *Cladosporium* - *C. herbarum* (Pers.) Link ex S.F. Gray, *C. cladosporioides* (Fres.) de Vries, *C. macrocarpum* Preuss - very common on senescent leaves.
8. *Curvularia inaequalis* (Shear) Boedijn - uncommon.
9. *Cylindrocarpon* sp. close to *C. orthosporum* (Höhnel) Woollenweber - very common in summer.
10. *Cylindrotrichum oligospermum* (Corda) Bon. - rare.
11. *Epicoccum purpurascens* Ehrenb. ex Schlecht. (syn. *E. nigrum* Link) - fairly common on senescent leaves.
12. *Fusarium*, special species - fairly common throughout the year.
13. *Geotrichum* sp. - uncommon.
14. *Helicodendron tubulosum* (Reiss) Linder - uncommon.
15. *Helicosporium* sp. - uncommon.
16. *Hormiactis* sp. close to *H. candida* Höhnel - repeatedly isolated in summer.
17. *Humicola grisea* Traaen - not very common, but recorded throughout the year.
18. *Microsporum* sp. - rare.
19. *Nigrospora* sp. - uncommon, but recorded throughout the year.
20. *Penicillium*, several species - very common in spring and summer.
21. *Phialophora* sp. - rare.
22. *Rhinocladiella* sp. - rare.
23. *Septonema* sp. - isolated repeatedly during summer.
24. *Stachybotrys atra* Corda - uncommon.
25. *Torula herbarum* (Pers.) Link ex S.F. Gray - uncommon.

26. *Trichoderma* sp. - common in spring, extremely common in summer.

27. *Trichothecium* sp. - uncommon.

28. *Tripospermum* sp. - uncommon.

29. *Scolecobasidium variabile* Barron et Busch - rare.

A hyphomycete which could not be identified by us, or by Dr. Ellis of the Commonwealth Mycological Institute and Dr. Gams of the Centraalbureau voor Schimmelcultures, was isolated repeatedly. It is referred to as UW 315.

(c) Coelomycetes

1. *Ascochyta* sp. - rare.

2. *Ceuthospora* sp. - repeatedly isolated in summer and spring.

3. *Colletotrichum* sp. - rare.

4. *Pestalotia* sp. - isolated several times in summer.

5. *Phoma* sp. - not uncommon on senescent leaves and throughout the field studies.

2. AQUATIC FUNGI

(a) Phycomycetes

1. *Pythium* sp. - appeared sometimes on leaves submerged in distilled water.

2. *Zoophagus insidians* Sommerstorff - fairly frequently observed on leaves submerged in distilled water.

3. *Acaulopage tetraceros* Drechsler - observed repeatedly on leaves submerged in distilled water.

(b) Hyphomycetes (distribution in different climates, based on reviews by Nilsson 1964 and Triska 1970, given in brackets).

1. *Actinospora megalospora* Ingold - rare (more common in tropics).

2. *Alatospora acuminata* Ingold - very common in winter, less common in summer (widely distributed, but usually more common in colder seasons).

3. *Anguillospora* - following Nilsson's (1964) suggestion, *A. pseudolongissima* Ranzoni was considered synonymous with *A. longissima* (Sacc. et Syd.) Ingold - common throughout the year (widely distributed).

4. *Articulospora tetracladia* Ingold - uncommon, only in summer (distributed throughout the world).

5. *Clavariopsis aquatica* De Wild - throughout the year, no seasonal pattern (widely distributed, more common in colder regions).

6. *Flagellospora: F. curvula* Ingold - not very common, throughout the year (widely distributed); *F. penicilloides* Ingold - rare (common in North America and in the tropics).

7. *Heliscus lugdunensis* Saccardo et Therry - very common in winter and spring (seems restricted to temperate and cold areas).

8. *Lemonniera aquatica* De Wild. - common in winter and autumn (concentrated in northern regions).

9. *Lunulospora curvula* Ingold - rare, only in summer (most common in tropical and subtropical regions).

10. *Tetrachaetum elegans* Ingold - rare, only in summer (most common in temperate regions, less common in cold and tropical regions).

11. *Tetracladium: T. marchalianum* De Wild. - common, mainly in winter and spring (more common in northern regions); *T. setigerum* (Grove) Ingold - less common (worldwide distribution, but usually not very common).

12. *Tricellula aquatica* Webster - rare (few reports).

13. *Tricladium: T. angulatum* Ingold - very common, especially at early stages of leaf decay, less common in summer; *T. gracile* Ingold - uncommon; *T. splendens* Ingold - uncommon (all three species probably more common in temperate zones).

14. *Triscelophorus monosporus* Ingold - rare, only in summer (main distribution in tropics).

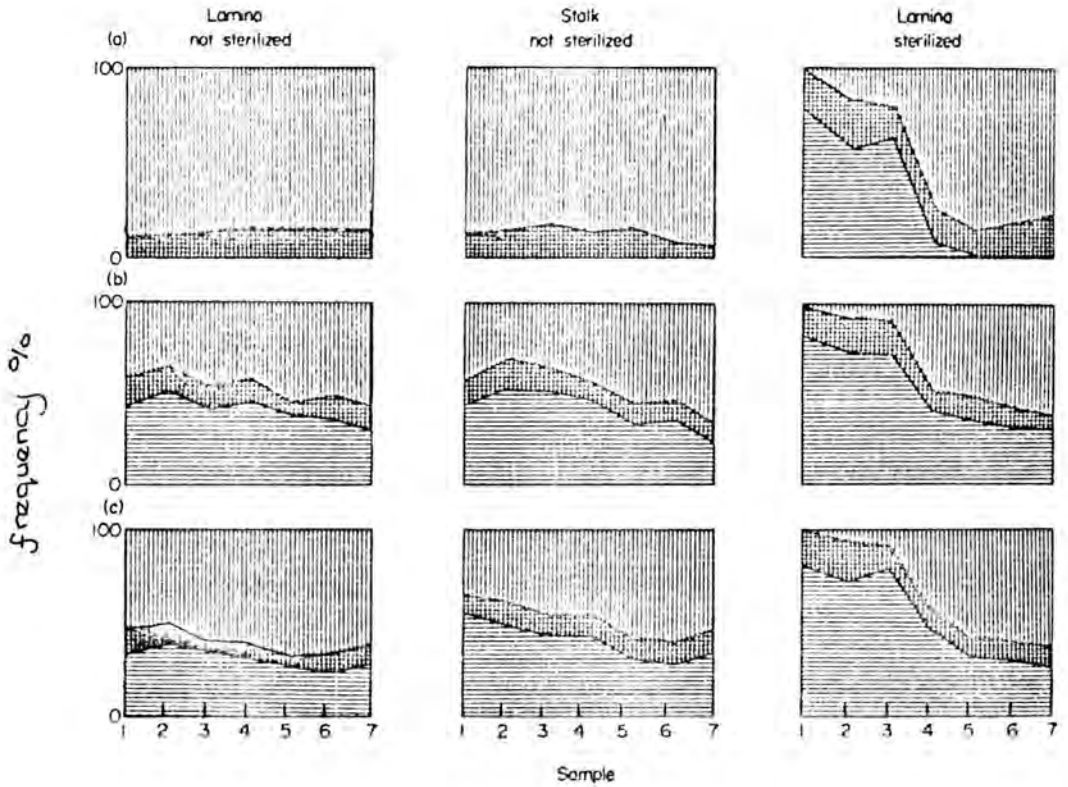


Fig 3.6 : Synopsis of isolation experiments : winter 1971-72

Incubation at <u>room</u> temperature

a) Malt extract agar

b) Water agar

c) Leaf agar

▥ frequency (%) of terrestrial fungi

▦ frequency (%) of sterile mycelia

▤ frequency (%) of aquatic fungi

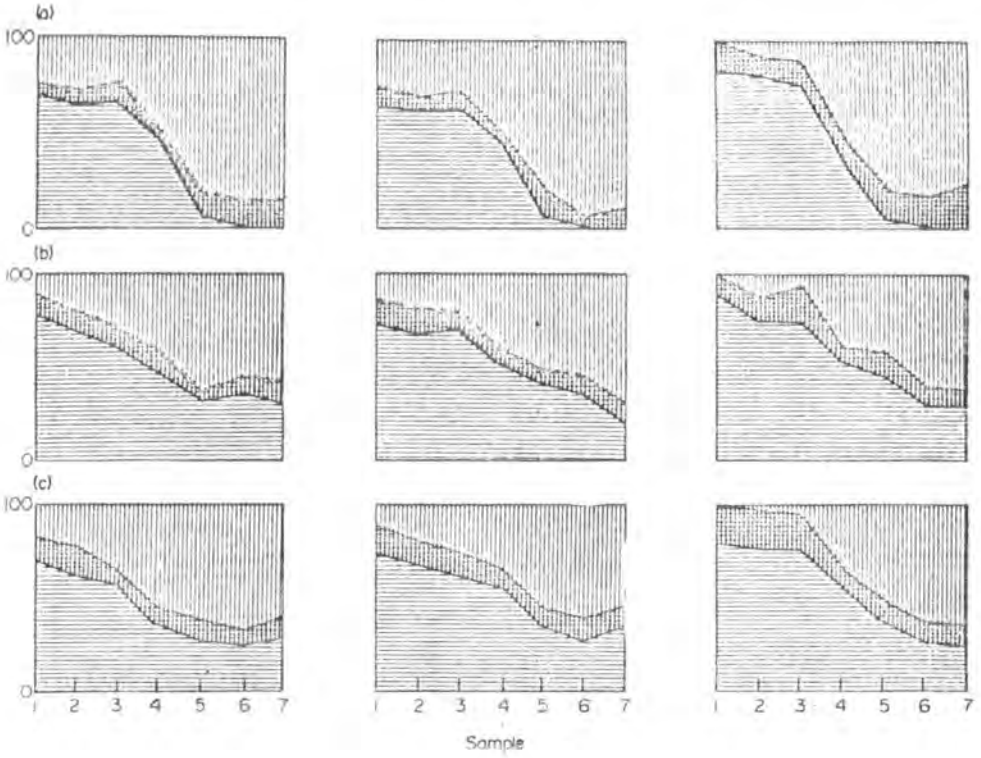
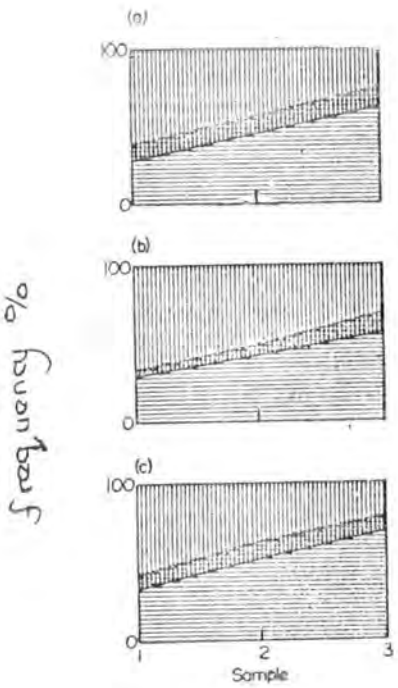


Fig 3·6 continued : Synopsis of isolation experiments winter 1971-72

Incubation at stream temperature.



Synopsis of isolation experiments
Winter 1972-73

All isolations on water agar
at stream temperature

a) Maple
b) Ash
c) Oak.

B & K presented the data from these experiments in three main ways:

First they listed all the fungi encountered on living, litter and submerged leaves with useful comments on how common or rare they were on these leaves, and whether or not their appearance was seasonal (see table 3.2). They made a convenient but slightly artifical split between those fungi regarded as terrestrial and those found mainly or exclusively in aquatic habitats. Apart from some aquatic phycomycetes observed only from the water incubations, the aquatic fungi were all aquatic Hyphomycetes.

Second, they gave detailed tables of the frequency of each species of fungus, isolated under each set of conditions, on each sampling date. Certain useful observations can be made from these results. For instance, the aquatic Hyphomycetes most commonly observed sporulating were Alatospora acuminata, Anguillospora longissima, Heliscus lugdunensis, Lemmoniera aquatica, Tricladium angulatum and Tetracladium marchalianum; whilst the terrestrial fungi which sporulated most abundantly were species of Alternaria, Aureobasidium, Cladosporium and Fusarium.

Third, they provided very clear summary diagrams, presented in Figure 3.6, which show the influence of leaf species, leaf parts, pre-sterilization, sampling data, incubation medium, and incubation temperature on the percentages of terrestrial, aquatic and sterile forms isolated.

The first field experiment provided information on the mycoflora on a single leaf species subjected to the whole spectrum of incubation conditions. To quote B & K'a clear and concise summary "It is immediately obvious that at the lower incubation temperatures more aquatic Hyphomycetes were isolated. At room temperature they could usually be isolated only on water agar and leaf agar. Aquatic forms appear to be less inhibited by low temperatures than terrestrial forms. Many of the terrestrial species were clearly already there before the leaves were

placed in the Speed River. On the sterile leaves they did not appear until the 3rd and 4th samples".

The way the terrestrial forms 'took over' and over ran the conventional medium at room temperature is very obvious from these clear diagrams. The contrast between incubations at room temperature and at the more appropriate stream temperatures is striking.

In the second field experiment, therefore, the incubation conditions were reduced to those known to be suitable for aquatic forms. They concentrated on looking for differences between the three leaves; a slow (oak), intermediate (maple) and fast (ash) decomposing species. The detailed frequency table showed that various differences did occur in the relative abundance of the various fungal species on these three leaves; essentially, however, their mycofloras were found to be very similar.

In the parallel laboratory investigations B & K's aim was to find out more about the environmental tolerances, colonizing and decomposing abilities of terrestrial and aquatic fungi recorded from the stream experiments. Such information might throw some light on the successional patterns observed; the main influences on succession being assumed to be stream temperature and leaf composition.

B & K had observed that "leaves freshly collected from the tree mostly bore propagules of terrestrial fungi such as Alternaria, Aureobasidium and Cladosporium. Yet after thirty five days in the stream Tricladium, Heliscus, Anguillospora, etc.,were more frequently isolated, at least on plates at stream temperature, suggesting that the terrestrial forms were being replaced by a new group, the aquatic Hyphomycetes".

They thus set up the following stream simulation experiments:

Leaf discs were cut from freshly fallen leaves, and from leaves retrieved from sample 1 (Jan), 2 (Feb), 3 (March), 4 (April) and 5 (May). These six sets of leaf material were inoculated, singly and collectively

with those aquatic and terrestrial fungi most frequently isolated from leaves of a similar condition. Thus the fungi recorded from sample 1 were inoculated onto fresh leaf discs, those from sample 2 onto leaf material from sample 1, etc. These discs were then incubated at the appropriate stream temperature.

The decomposition ability of each fungal species was reflected in weight lost from the disc, whilst the amount of fungal biomass elaborated was indicated by increases in leaf protein content.

These experiments produced useful and interesting data but B & K were cautious in reading too much into them. The aquatic Hyphomycetes were more active than the terrestrial fungi at the low temperatures (leaf samples from Jan, Feb, March and April). This was reflected in significant weight losses and significant increases in protein content. In the 'warmer' sample however the situation was less clear. Some of the terrestrial fungi were more active whilst the aquatic were less active. This tied in with the larger proportion of terrestrial fungi observed at higher stream temperatures.

Simultaneous inoculation of fungi into one leaf disc consistently produced higher weight loss and increase protein content than any single fungus, terrestrial or aquatic (at least in the first four samples). This suggested that the fungi, principally the aquatic Hyphomycetes dominant in these samples, were acting together to decompose the leaf material. This ties in with Ingolds' observation that older leaved bearing aquatic Hyphomycetes had an average of five different species, all growing together. This may indicate some subtle division of labour. Certainly Heliscus lugdunensis, one of the commonly observed aquatic Hyphomycetes, tended to grow on the petiole and on viens whilst Tricladium angulatum was usually found on the lamina.

Another interesting observation from these laboratory experiments was that increases in leaf protein content sometimes occurred without

a parallel weight loss. This suggested that the fungi had exploited external sources of nutrients, rather than leaf material, in increasing their biomass. (see 3.2.3)

In their conclusions and discussion B & K stress again the difficulty of assessing the activity of a fungus when the laboratory methods used in isolation and identification may radically alter what was actually happening on the leaf: the difficulty in distinguishing "between metabolically active organisms which play a part in the degradation process and those which are passively present in a dormant or inactive form".

Many investigators have found aquatic Hyphomycetes to be the dominant fungi sporulating on leaves retrieved from streams. Such sporulation in situ is a very good indication of activity, and suggests they may be the main agents of decomposition of these leaves. Bärlocher and Kendrick's 'particle-plate' incubations from their field samples showed that these fungi were common on leaf and water agar, as well as all leaf material incubated in water. These conditions reflected stream conditions more accurately than the other incubation regimes and the mycoflora revealed is more likely to reflect the true situation.

Their conclusions are best summarized by this quote: "Because of these potential pitfalls the description and interpretation of the fungal succession on leaves is inherently tentative. When the leaf is still hanging on the tree, it already carries many propagules of several fungi; (Alternaria, Cladosporium, Aureobasidium etc.). Depending on whether the leaf lands directly in the stream or spends some time on the ground beforehand, and on the prevailing conditions, these fungi may rapidly colonize the leaf. In the stream, the temperature will eventually fall to low values which will at least partly inactivate many common soil

fungi, while the aquatic species can still colonize and degrade the leaf. Aquatic Hyphomycetes predominate throughout the colder months until higher spring temperatures allow renewal activity of the geofungi still present, and also permit new infections by other fungi. In summer, geofungi may be as important as - perhaps more important than - the aquatic species. Nevertheless, the main factor which decided which fungi would be dominant in the early succession on the leaves used in the present study... seems to have been ability to grow at very low temperatures".

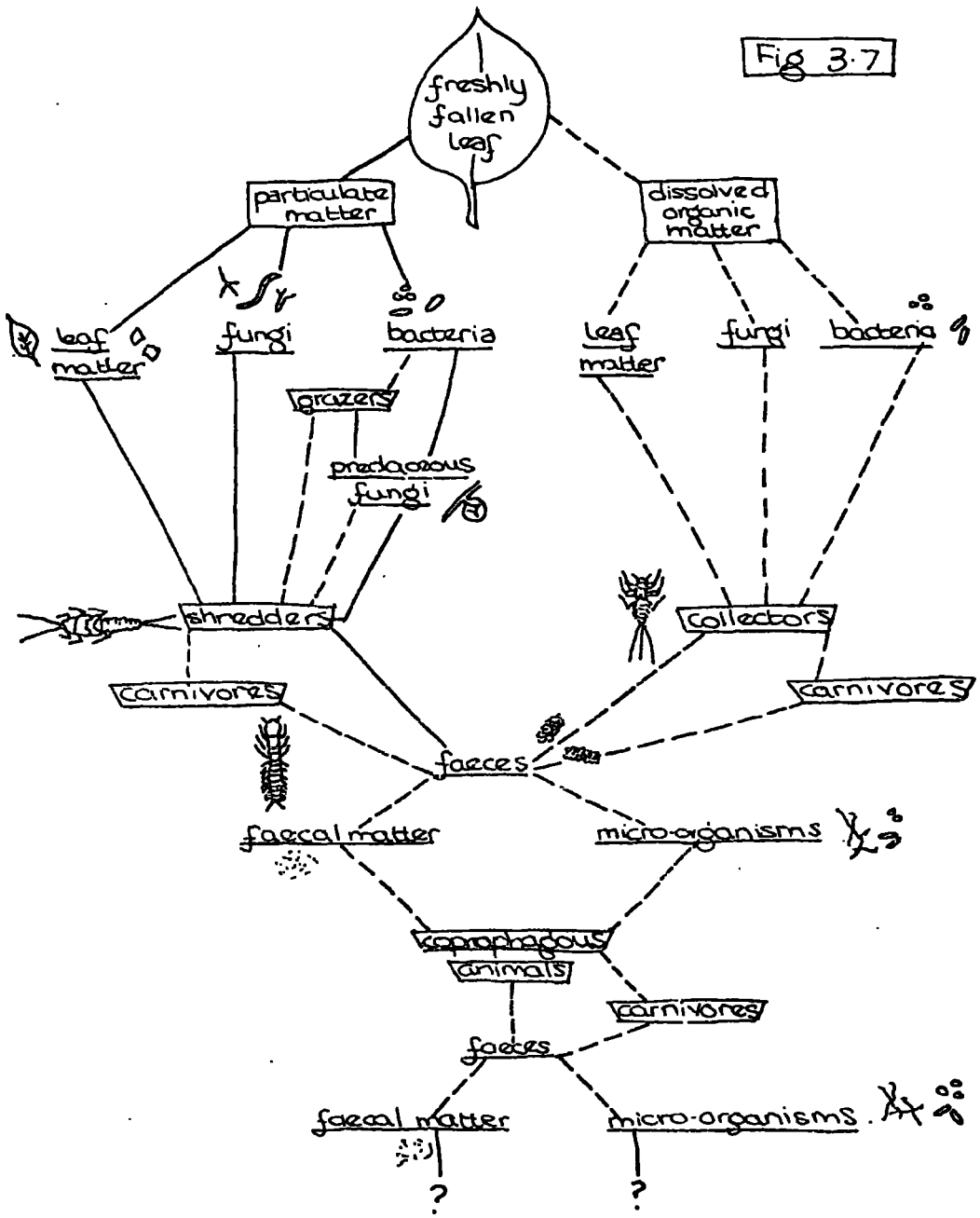
They discuss the obvious success of aquatic Hyphomycetes in well aerated streams and conclude that this is related mainly to spore form, with a subsequent adaptation to the particular environmental influences important in various geographical locations - such as autumn leaf fall and low stream temperatures in temperate streams.

Their discussion continues with an assessment of the role of fungi in introducing "the allochthonous leaf and its potential into the energy flow of the stream community"(and illustrate)their ideas in a scheme shown in figure 3.7 (see also 3.4).

3.3.3 Suberkropp and Klug's study

Suberkropp and Klug faced the same difficulties as Bärlocher and Kenderick in relating the frequency of isolation of various members of the leaf microflora in the laboratory to their activity on the decaying leaf in the stream.

They also decided to use a spectrum of incubation conditions. However the methods they used, both in the observation and recording of the fungi found, were rather more sophisticated; revealing more about the relative activities of the various fungi found and providing detailed information on the successional patterns of dominant aquatic



Presumptive steps in the incorporation of the energy represented by autumn-shed leaves into stream communities.
After Bärlocher & Kendrick 1974
—— observed in Bärlocher & Kendrick's studies (1973a, b)
--- not observed in their studies.

The fate of the insoluble and soluble leaf matter is not necessarily as strictly separated as the figure implies
e.g Micro-organisms on the leaf (e.g. aquatic Hyphomycetes) may absorb dissolved organic material from the water. Their activity may lead to the release of soluble substances. Collectors may also "collect" leaf fragments produced by physical fragmentation or undigested in faecal pellets.

Hyphomycetes.

In explaining their interest in the composition and role of the mycoflora of submerged autumn-shed leaves S & K stress the observed dominance of aquatic Hyphomycetes on such leaves. They point out the lack of information available on the physiological capabilities of these fungi; information that would reveal much more about their role in leaf processing.

They also discuss the rather ambiguous presence of terrestrial fungi in streams: "other types of fungi, chiefly those associated with terrestrial communities, have also been observed on leaf litter in streams... Data are scarce on the role of these fungi in streams, but recent evidence indicates that they are probably not active during the periods of low temperature encountered during the winter in temperature climates and that their presence may be due to colonization of the senescing leaves before abscission (B&K) or to passive entrapment of dormant propagules on the leaf substrata in streams (Park 1974)".

In their field study programme S & K collected the leaves of oak and hickory as they were shed from the trees; they then assembled them into leaf packs before placing them in the stream "in a manner similar to natural leaf accumulations".

Between November and June, bi-weekly samples of leaves were retrieved from the stream; and small discs (0.5cm diameter) were cut out of these leaves for mycological observation.

Five techniques were used "to assess the types of fungi present on the leaves". The details of these techniques are given in tables 3.3 and 3.4. They include a direct observation technique to detect any fungi sporulating in situ; two low temperature incubations in water and on an inorganic salts medium (ISA) (conditions likely to favour the growth and sporulation of aquatic Hyphomycetes); and two high temperature

TABLE 3.3

SUBERKROPP AND KLUG'S STUDY: FUNGAL OBSERVATION TECHNIQUES

Direct

Leaf disks were fixed immediately in lactophenol; 30 disks were later stained with lactophenol-cotton blue and the entire surface of each disk scanned at 160X to determine sporulating fungi.

Water Incubation

Leaf disks were incubated in 250 ml of aerated, filter-sterilized (0.45 µm membrane) stream water. The duration and temperature of the incubation were varied with natural stream temperatures, i.e. 4 days at 5°C during November through March, 3 days at 10°C during April and May, and 3 days at 15°C during the latter part of May and June. After incubation, disks were fixed in lactophenol, and 30 were later stained and examined as in the "direct" technique.

Low temperature inorganic salts agar (ISA) incubation

After disks were incubated on ISA (KNO_3; 2.5g; K_2HOP_4, 3.4g; $MgSO_4 .7H_2O$; as in the "water incubation", 20 disks were stained and examined as in the "direct" technique.

High temperature ISA incubation

After disks were incubated on ISA at 25°C for 6 days, 25 disks were examined at 50X for sporulating fungi.

Particle plate incubation

Disks were incubated in a starch-casein-nitrate medium with 0.035% rose bengal (Ottow 1972) at 25°C for 6 days. Sporulating fungi on 25 disks were determined as in the "high temperature ISA incubation" technique above.

Table 3.4

Name of technique	Incubation medium	Temperature	Incubation period	Further treatment
*Direct observation	-	-	-	Leaf disks fixed immediately; later stained and scanned at X160 for sporulating fungi
*Water incubation	Aerated, sterilized stream water	5°C for	4 days (Nov-Mar) 3 days (Apr-May) 3 days (May-June)	as above
*Low temperature inorganic salts agar (ISA)	ISA	as above	as above	as above
High temperature inorganic salts agar (ISA)	ISA	25°C	6 days	disks examined at X50 for sporulating fungi
Particle plate incubation	Starch-casein-nitrate medium	26°C	6 days	As for high temperature ISA

*See Section 4.2

incubations, one on the salts agar the other on a rich organic medium.

Between them such a range of techniques is likely to reveal most members of the leaf mycoflora - be they aquatic or terrestrial - and present as active hyphae, inactive hyphae or spores.

They also used a more detailed recording method for the fungi observed. Not only were percentage frequencies calculated for each fungus from each technique, but the density of each fungus on each leaf disc was scored on a scale 0 to 3 (for the first three techniques). This scale is given in table 4.5. These frequency and density records were then combined to produce 'importance indices' which gave ecologically interesting information on the relative importance of each species.

In B & K fungal biomass was estimated by measuring hyphae length per unit area of leaf. S & K, concerned with the combined fungal and bacterial population, decided to use ATP concentration as an indication of active microbial biomass in the leaves being processed. In Suberkropp et al (1976) they followed the progression of microbial colonization and decay by measuring changes in the soluble and non-soluble components of leaves - changes in cellulose and protein content, for instance.

Assessing the frequencies of various fungi recorded from the first three 'observation' techniques, S & K found that six species of aquatic Hyphomycete emerged as major colonizers of leaf material. These were:

Flagellospora curvula

Lemmoniera aquatica

Alatospora acuminata

Tetracladium marchalianum

Anguillospora sp

Clavariopsis aquatica

Many other workers have found these species to be abundant in cold and temperate streams in both North America and Europe. (see table 2.6).

The direct observation technique in this study showed that these aquatic Hyphomycete species were sporulating *in situ* on the leaf when it was removed from the stream. S & K and others, including Park (1974), view this "as strong evidence that these fungi are growing in this environment, and therefore, are active in the processing of the leaves". Many other people have demonstrated aquatic Hyphomycete sporulation on freshly collected submerged leaves. For instance, S & K in an earlier paper (1974), revealed abundant sporulation of aquatic Hyphomycete on the surface of such leaves by the use of the scanning electron microscope.

Although fungi sporulating *in situ* on newly collected leaves are likely to be active, the converse is not necessarily true. Fungi not sporulating may be active; they may be 'concentrating' on vigorous vegetative growth. Such fungi will be capable of sporulating fairly rapidly if encouraged. Hence the use of laboratory incubation; sporulation is stimulated, identification is possible and density can be better assessed.

Of the four incubation regimes used by S & K, two "indicated the same species composition as direct examination, but produced higher frequencies and densities of sporulation". These were the water incubation, and the low temperature ISA - the two regimes combining environmental incubation temperatures with short incubation periods. Only fungi which sporulated rapidly would be shown up by these regimes. Active fungi would be capable of such rapid sporulation and Park (1974) suggests that this ability is a good indication of activity.

Table 3.5 gives the importance indices of the six common species of aquatic Hyphomycetes over the November to June sampling period. Differences in abundance and successional pattern between the two leaf

	Weeks															
	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32
Hickory																
Flagellospora curvula	1.38	1.29	0.41	0.27	0.34	0.08	0.14	0.05	0.01	0.02	0.01					
Lemonniera aquatica	0.60	0.23	0.20	0.24	0.21	0.32	0.13	0.25	0.21	0.44	0.29					
Alatospora acuminata	0.00	0.13	0.71	0.63	0.50	0.63	0.60	0.46	0.61	0.56	0.75					
Tetracladium marchalianum	0.00	0.00	0.32	0.38	0.25	0.62	0.54	0.77	0.62	0.67	0.55					
Anguillospora sp.	0.00	0.12	0.18	0.31	0.26	0.19	0.44	0.36	0.35	0.21	0.35					
Clavariopsis aquatica	0.00	0.00	0.09	0.07	0.11	0.15	0.12	0.08	0.21	0.05	0.01					
Others	0.02	0.00	0.07	0.09	0.32	0.01	0.04	0.02	0.00	0.04	0.04					
Oak																
Flagellospora curvula	1.13	0.94	0.84	0.42	0.40	0.20	0.17	0.33	0.14	0.02	0.05	0.00	0.00	0.00	0.00	0.00
Lemonniera aquatica	0.75	0.78	0.66	0.41	0.43	0.34	0.48	0.29	0.49	0.43	0.46	0.32	0.28	0.11	0.16	0.00
Alatospora acuminata	0.12	0.12	0.28	0.68	0.48	0.60	0.57	0.64	0.74	0.92	1.03	0.78	1.16	1.45	1.52	1.76
Tetracladium marchalianum	0.00	0.00	0.02	0.15	0.07	0.08	0.18	0.38	0.12	0.20	0.04	0.18	0.17	0.00	0.00	0.00
Anguillospora sp.	0.00	0.02	0.05	0.20	0.17	0.19	0.20	0.20	0.15	0.22	0.21	0.31	0.17	0.26	0.21	0.12
Clavariopsis aquatica	0.00	0.02	0.14	0.09	0.44	0.48	0.36	0.15	0.36	0.20	0.19	0.38	0.16	0.18	0.05	0.12
Others	0.00	0.12	0.02	0.05	0.01	0.01	0.04	0.00	0.00	0.00	0.01	0.03	0.06	0.00	0.05	0.00

Table 3.5

Importance Indices

Suberkropp & Klug 1976.

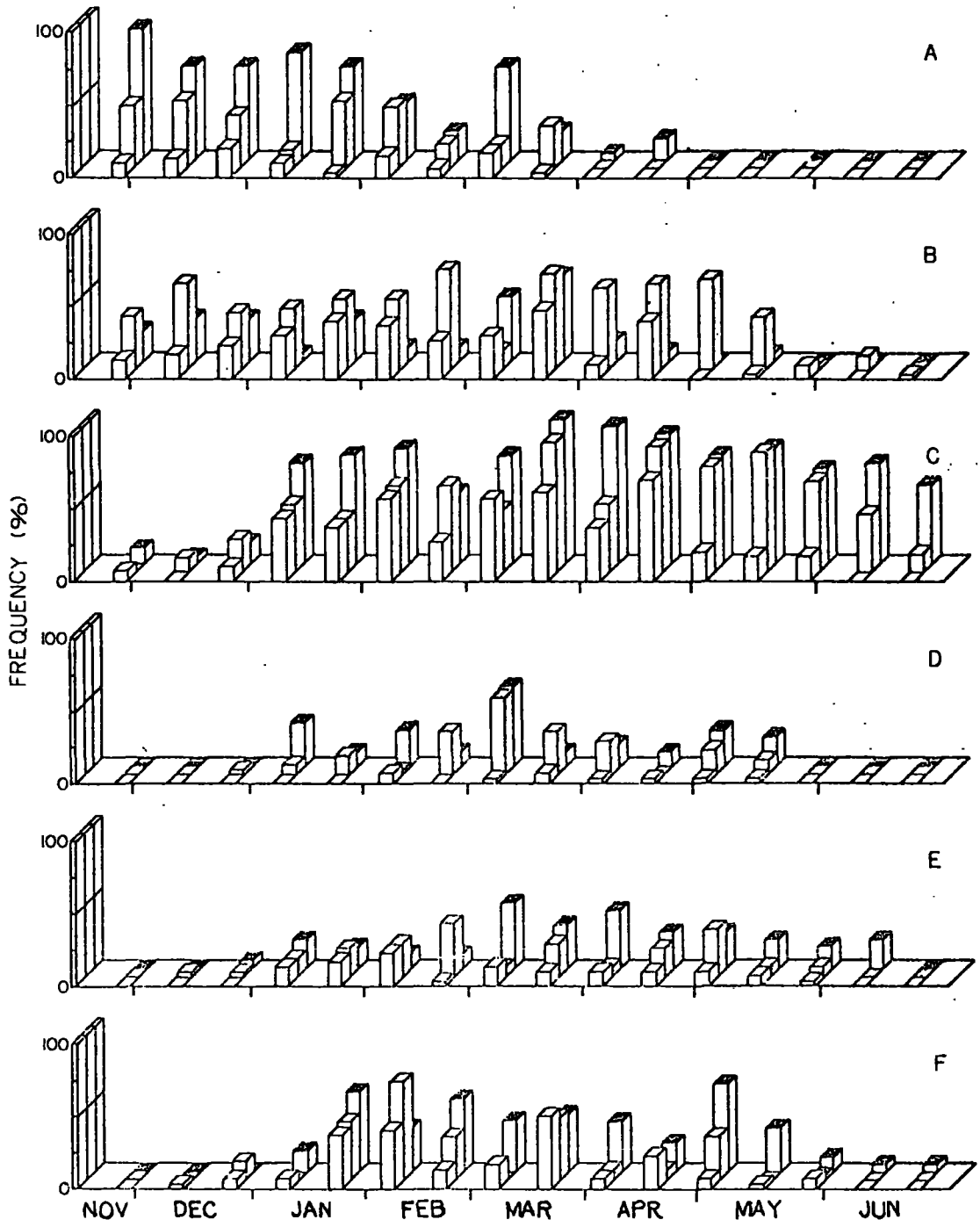


Fig. 3·8 Occurrence of aquatic Hyphomycetes on oak

A	F. curvula	D	T. marchalianum
B	L. aquatica	E	Anguillospora sp
C	A. acuminata	F	C. aquatica

white bars : frequencies determined by direct examination
grey bars : frequencies determined after water incubation
black topped bars: frequencies determined using low temperature ISA incubation

Suberkropp & Klug 1976.

species can be seen; the slower development and longer "life" of the aquatic Hyphomycete flora on the oak leaves is particularly striking. However, the same six species occur as major colonizers on both leaves.

Figure 3.8 shows S & K's excellent 3D histograms illustrating the frequencies of these six species of aquatic Hyphomycete on oak leaves over the seven month sampling programme. These clearly show the successional patterns within the mycoflora; for instance, the initial colonization of oak by F. curvula, and the dominance of A. acuminata. They also show the differences in frequency revealed by the three 'observation' techniques.

How do S & K explain these successions? They suggested that the high PH of the water and the low stream temperatures were responsible for the decline in the terrestrial fungi introduced with the leaf, and helped determine the dominant aquatic Hyphomycete species. What influenced the pattern observed for these six species?

If the different species had different enzymatic capabilities then changes in the composition of the decaying leaf would bring about changes in the balance of colonizing fungi. However there is no evidence for such differences, on the contrary the information provided in this paper suggests that all these species are capable of breaking down the major structural components of hickory leaves, at least.

What other influences could affect succession? Metabolic interactions, both stimulatory and inhibitory, can occur between fungal and bacterial members of the leaf microflors, affecting competition and exploitation of the leaf material. However details of the effect of such interactions in this study are not known.

Thus S & K could not come to any firm conclusions on the successional patterns they revealed "clearly additional information on the physiological and competitive capabilities of these fungi is needed to

fully understand their role in the processing of leaf litter in woodland streams.

S & K then considered the fungi revealed by the two remaining 'observation' techniques. These incubations were at higher temperatures and left for longer before observation took place. The results were very different, the leaf material being covered with sporulating soil and litter fungi. Such species had been reported from streams before, by several workers. In each case similar plating techniques had been used to stimulate sporulation.

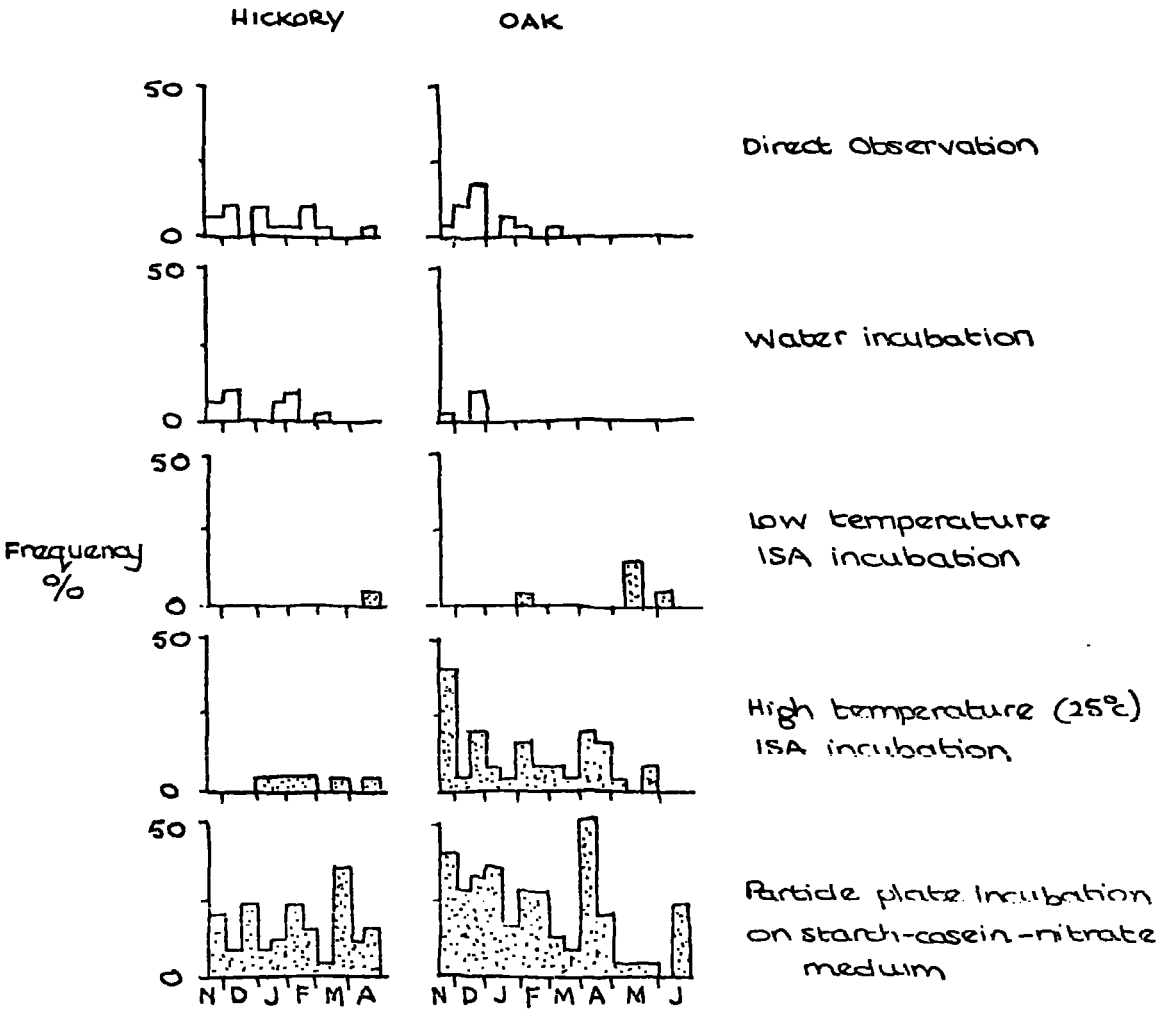
Figure 3.9 shows the occurrence of Alternaria sp. - a common phylloplane and leaf litter genus - under the different regimes. The contrast between the practical absence of sporulation in the first three techniques and the profuse sporulation in the rich SCN particle plate incubation is striking. Similar results were obtained for the other terrestrial fungi except that only Alternaria Fusarium and Cylindrocarpon were observed sporulating on anything but the SCN. These fungi showed no successional pattern in contrast to that of the aquatic Hyphomycetes revealed by the other three techniques.

"In order to determine which of the two series of results is representative of the fungal populations active in the environment the conditions used in each incubation must be critically evaluated".

The two 'sets' of incubation regime differed in three main ways. The first (water and low temperature ISA) employed unenriched media for short term incubation at stream temperatures. The second set (high temperature ISA and SCN) employed a rich medium (SCN) for longer term incubations at high temperatures.

Which of these two sets was most likely to reveal the 'true' leaf mycoflora active in decomposition?

Both Hudson (1968) and Park (1974) have suggested that short



Occurrence of *Alternaria* sp. on oak and hickory

after Suberkropp & Klug 1976.

□ frequencies for spores only

▨ frequencies of sporulation.

Fig. 3.9

incubation periods are desirable; these allow fungi just about to fruit to do so without encouraging spores and other resting, inactive, structures to germinate grow and fruit.

Kaushik and Hynes (1971) found mainly terrestrial fungi growing on their incubation plates. They used conventional laboratory media (and incubation conditions) which differed considerably from the natural substrate, and which could have encouraged the vigorous growth of fungi which would not have thrived on the leaf when it was in the stream.

Incubation temperature is also rather important. Obviously an 'environmental temperature regime' reflects field conditions more accurately - not only do incubations at 'standard room temperature' encourage inactive fungi to grow, they may also inhibit fungi active at the lower stream temperatures. Thus S & K observed that aquatic Hyphomycete growth was very poor at 25°C. Such slow sparse growth could quickly be swamped by 'weed' fungi. B & K had pointed out the higher frequency of aquatic fungi revealed by incubation conditions which reflected the stream environment.

It does seem sensible to expect such incubation conditions to more accurately reveal the true leaf mycoflora. Certainly the emergence of the aquatic Hyphomycete species as important members of the mycoflora (revealed by the first three techniques) agrees with many previous observations on their abundance on autumn shed leaves. It is safe to assume that these fungi are in fact active in the stream environment and that the environment simulating incubation regimes are the best to use when surveying such stream floras. The contrast between the two mycofloras revealed by the two sets of incubation regime stresses just how different a picture is gained by using the sort of media and incubation conditions common in the maintenance, growth and manipulation of pure cultures of fungi in the laboratory.

Thus although terrestrial fungi have been recorded from leaves in streams* S & K concluded that, at least during their sampling programme, these fungi were inactive. The lack of any successional pattern shown by these fungi contrasts with the definite successional patterns of the aquatic Hyphomycetes which in fact "agree very well with the relative rates at which the two leaf species are processed".

S & K draw together the threads of their laboratory and field investigations in the form of a scheme for the initial stages of autumn-shed leaf processing:

"The data presented in the present study, as well as those of Triska (1970) and Kaushik and Hynes (1971), provide strong evidence that fungi dominate the microbial biomass during the initial phases of processing of oak and hickory leaves in temperature streams. Bacterial biomass increases during the decline of fungal biomass and dominates the microflora in the terminal stages of processing".

Fungi, in this case aquatic Hyphomycetes, are particularly suited to initial colonization of the leaf because of their invasive growth and range of extracellular enzymes. Unpublished data suggest that all the aquatic Hyphomycetes are capable of producing pectinase and cellulose This initial fungal colonization and breakdown of cell structure and cell components conditions the leaf for invertebrates; and combined fungal and invertebrate maceration the tissue conditions the leaf for the bacteria: "greatly increas(ing) the colonizable surface area of leaf material and presumably releas(ing) previously bound plant constituents".

Fungal conditioning must proceed increased bacterial colonization because few of the bacteria can degrade structural polymers.

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* Park in his 1972 paper "On the ecology of heterotrophic organisms in freshwater" discusses the origins, activity and detection of terrestrial fungi in freshwater habitats.

Bacteria are also less active during the initial stages of processing because of low stream temperatures to which the aquatic Hyphomycetes are adapted. However unpublished work on leaf processing in the summer, when stream temperatures are more favourable to bacterial growth, shows that fungi still dominate these early stages.

Differences in microbial biomass associated with oak and hickory are likely to be due to leaf chemistry. Probably the complexing of proteins with phenolic compounds in the oak makes them less available to microbal decomposers. This would explain the slower processing rate observed in oak leaves (see 3.2.3).

To conclude: this careful and comprehensive paper combines extensive laboratory and field work on the occurence and capabilities of the fungi and bacteria found on autumn-shed leaves. Not only is the role of aquatic Hyphomycetes in leaf decomposition further confirmed and explained but the authors make a valuable contribution to fungal ecology in their use and discussion of environmentally appropriate laboratory conditions.

3.4 Hyphomycetes as intermediaries of energy flow in streams.*

3.4.1 Introduction

Several ecological observations have led to the current interest in the ability of aquatic Hyphomycetes to increase the attractiveness and food value of leaves to stream invertebrates.

Most streams are heterotrophic with a food chain based mainly on autumn shed leaves. These are "known to serve as food for many members of almost all groups of benthic [or bottom dwelling] organisms". However, although these leaves are such an important food "only a small fraction of the energy represented by the leaf material can be directly exploited by animals". For instance, no animal, except with the symbiotic assistance of gut microflora, is capable of breaking down cellulose and lignin for their contained energy.

Bacteria have long been known to 'intervene' in aquatic and terrestrial detrital food chains. For instance: the fine organic particles consumed by filter feeders as they sort through estuarine silt deposits are enriched with nitrogen by colonizing bacteria. The nitrogen is assimilated along with some of the carbohydrate but much of the material is passed out as faeces where its nitrogen content is again raised by re-colonization by bacteria.

However, fungi are also important in the colonization and break-down of plant material in both aquatic and terrestrial habitats. Aquatic Hyphomycetes are particularly conspicuous as colonists of submerged autumn-shed leaves. "The ecological significance of fungi in the food chain in aquatic environments, especially streams, has been ignored until recently. This neglect is all the more suprising when one reflects that fungi are superior to bacteria in their ability to degrade cellulose and lignin... substances which although they account for 34-65% of the dry weight of

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* Many of the quotes in this section, unless otherwise indicated derive from the useful Barlocher & Kendrick review of the same name.

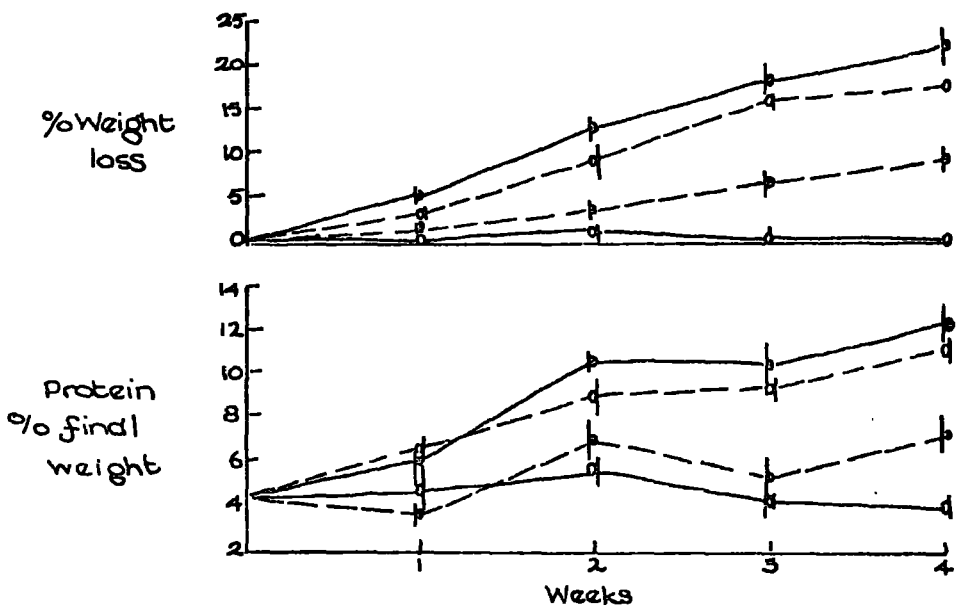
leaves cannot be digested by most invertebrates". Much of the material is passed through undigested, the assumption being that the animal has assimilated a small, easily digested fraction of the food, probably mainly microbial material. The energy rich faeces that remain pass on to another stage of decomposition, with colonization by a different range of fungal and bacterial species and consumption by the 'collector' invertebrates (see Figure 3.4) The cycles repeats itself until the nutrients in the leaf material have been exploited by the combined activities of micro-organisms and invertebrates.

3.4.2. 'Conditioning' of leaves by aquatic Hyphomycetes.

Several recent studies have attempted to find out whether colonization by fungi, in particular by aquatic Hyphomycetes, contributes to the acceptability of autumn-shed leaves as invertebrate food.

Kaushik and Hynes (1968, 1971) and Triska (1970) appear to have stimulated much of the recent work. Kaushik and Hynes observed increases in protein content of leaves in streams as they decomposed. Such increases were significantly depressed by the use of antifungal antibiotics and were thus attributed to fungal activity (see Figure 3.10). Experiments by both Kaushik and Hynes, and Triska indicated that the fungal contribution to leaf degradation was much greater than the bacterial contribution. They also carried out 'preference experiments' in which invertebrates could choose between different species of leaf with or without colonizing fungi and bacteria. The results showed that several stream detrivores preferred to eat partly decomposed leaves with a rich microbial population rather than sterile or freshly fallen leaves. In Triska's experiments this population was largely aquatic Hyphomycetes.

How can aquatic Hyphomycetes affect the value and acceptability of the leaf as invertebrate food? There appear to be three main ways: the



Decomposition of elm leaves. % weight loss and protein content of leaves kept at 10°C in stream water with different antibiotics Mean values • ± 95% confidence limits ⊢

control A
antifungal + antibacterial
antifungal
antibacterial

After Kaushik & Hynes 1971

Fig 3·10

conversion of existing leaf material to fungal biomass; the 'trapping' of soluble nutrients from the surrounding water to augment the leaf's resources; and the breakdown of cell structure and molecules without assimilation into fungal biomass.

The evidence is that aquatic Hyphomycetes are particularly versatile in their enzymic capabilities and can convert both simple and complex carbohydrates to fungal biomass. Their dominant presence on leaves throughout much of the year also suggests that they have the opportunity to use these abilities.

Bär locher and Kendrick (1974) have demonstrated an increase in the nitrogen content of aquatic Hyphomycete colonized leaf discs, without a parallel decrease in leaf weight, which they attribute to nitrogen trapping from the surrounding water. The fungi appear to augment the already severely depleted and decreasing nitrogen supply of the leaves themselves, making the C:N ratio more favourable to decomposition.

Fungal degradation of substrates involves the excretion of exoenzymes and then the absorption of the breakdown product, perhaps the simple carbohydrates produced by the hydrolysis of more complex molecules such as cellulose. Not all of the breakdown product will necessarily be absorbed. Suberkropp and Klug (1976) suggest that all aquatic Hyphomycetes "are capable of elaborating pectinase" and enzyme which will disrupt the cell structure by dissolving the middle lamellae.

The 'conditioned' leaf preferred by aquatic and terrestrial invertebrates is thus an aggregate of unchanged leaf material, partly broken down leaf material, microbial cells (in this case principally aquatic Hyphomycete hyphae) and microbial excretions and secretions.

3.4.3 Consumption, assimilation and preference experiments.

A number of experiments have been done on the consumption, assimilation and attractiveness of diets consisting of leaves without

colonizing fungi, pure cultures of fungi, and leaves with their compliment of fungi.

Bärlocher and Kendrick, in their 1973 papers, assessed the efficiency with which a freshwater shrimp Gammarus psuedolimneaus converted leaf and fungal material into its own biomass. Increase in body weight and daily food consumption were measured.. ...The diets provided and the results gained are shown in Figure 3.11.The highest weight increases were shown by shrimps feeding on four fungal diets - three of these being aquatic Hyphomycetes. Also much larger quantities of leaf material as opposed to fungal material had to be consumed to produce the same weight increase. The fungi thus provide a much more concentrated food. These experiments indicate that certain aquatic Hyphomycetes are a good food source in themselves, whilst " more significantly, we can confidently assert that good fungal growth on the leaves will substantially improve their food value to the invertebrate stream fauna".

In 1975 Bärlocher and Kendrick followed up these experiments with a study on the actual assimilation, rather than just consumption,of the same range of diets. The results lead the authors to substantially the same conclusion.

The significance of these results may be different for scrapers, which remove the top layer of colonizing micro-organisms and leaf material from the surface of the leaf; and for shredders which consume 'chunks' of the leaf. (Gammarus are shredders).

The value of an enriched food source is obviously enhanced if the consumers prefer it to less valuable sources. Many workers have carried out more or less sophisticated preference experiments using leaves with and without their microflora as well as pure cultures of fungi and bacteria. Bärlocher and Kendrick in their 1976 review report a food selection experiment in which shrimps were offered a choice between various combinations of three leaf species and pure cultures of the fungi listed in Figure 3.11 (5 of them aquatic Hyphomycetes). The results show a

Assimilation of fungus and leaf by Gammarus. All values milligrams dry weight per animal per day.

after Bärlocher & Kendrick 1976

Fig 3·11

difference in preference between the fungi, all of which were more attractive than maple leaves. Offered leaf material only, the shrimps preferred ash > maple > oak. They add a caution at this point "such strict segregation of leaf and fungus is unlikely in nature and we must emphasize that the normal feeding mechanism of Gammarus involves ingestion of the leaf together with the fungal population it bears."

They went on to study how individual species of fungi influence the palatibility of the leaves they colonize. Discs of each leaf species were inoculated with pure cultures of the different fungi and then offered to the shrimps in various combinations. The results showed that the normal order of preference ash > maple > oak can be altered. In essence, the most palatable fungus colonizing the least attractive leaf can make this leaf the first choice of a shrimp also offered the most attractive leaf with the least palatable fungus - and all the variations in between. Particularly interesting was the fact that Anguillispora, the aquatic Hyphomycete which produced the greater weight increase in the consumption experiments was amongst those fungi which made the leaves most attractive.

Bärlocher and Kendrick assess these results in the light of the ecological role of fungi and invertebrates in the processing of leaf material. "[These results] clearly demonstrate the decisive influence which the fungal population can have on food selection by Gammarus, and presumably other stream invertebrates, [and] suggests that the various leaf-fungus substrates fall along a graduate scale, from those which are eagerly eaten as soon as they become available, to those which are tardily and reluctantly consumed. This suggests a natural rationing or regulatory process which may help the large winter populations of benthic invertebrates to survive by spreading out their consumption of available food over a longer period than would be dictated by their

natural appetites if all food materials were equally palatable". Since aquatic Hyphomycetes are the dominant mycoflora on submerged autumn-shed leaves they will be the ones to provide the "decisive influence" on food selection in streams.

Willoughby and Sutcliffe (1976) and Marcus and Willoughby (1978) assessed the food value of a variety of natural and artifical diets to the shrimp Gammarus pulex and the water hog-louse Asellus aquaticus. They found that naturally decaying deciduous tree leaves - with their compliment of organisms, including aquatic Hyphomycetes - provided the best diet in terms of growth and survival. Thus aquatic Hyphomycetes are important to invertebrates and the combined activities of both are important in the ultimate retrieval of the energy and nutrients locked in leaves. Conversely, the importance of autumn-shed leaves to both aquatic Hyphomycetes and invertebrates is illustrated by the adaptation of aquatic Hyphomycetes to vigorous growth and sporulation in the cool stream temperatures which prevail when leaf material is most plentiful (Bärlocher and Kendrick 1974): and by the fact that "A large portion of the aquatic insect community has become synchronized to the autumnal input of leaf material". (Peterson and Cummins 1974). The concluding remarks of Bärlocher and Kendricks review provide a succint summary of, and final comment on, this interesting area of study: "our own experiments and other reports have clearly demonstrated that the fungi, especially the typical freshwater hyphomycetes, are invaluable members of the stream community and can no longer be neglected in any comprehensive account of life in running waters. They play a key role in unlocking and distributing a major source of energy in streams. This is a very new and wide open field; we look forward to an extremely productive period, and we hope that this summary of current research will encourage more ecologically oriented mycologists to 'get their feet wet'."

4. METHODS IN THE STUDY OF AQUATIC HYPHOMYCETES

4.1. Techniques used in the study of aquatic Hyphomycete biology and ecology

4.2. A summary of the Field Study Programme: Aims, Scope and Construction

4.3. Methods and Materials

4.3.1. Sample Sites

4.3.2. Field methods

4.3.3. Laboratory methods: preparation of materials

4.3.4. Laboratory methods: observation and recording

4. METHODS IN THE STUDY OF AQUATIC HYPHOMYCETES

4.1 Techniques used in the study of aquatic Hyphomycete biology and ecology

In considering the various techniques used in the study of aquatic Hyphomycetes it is useful to consider the following questions:

What different aspects of the biology and ecology of aquatic Hyphomycetes interest the laboratory and field investigator?

What particular problems occur in the collection and manipulation of field and laboratory material: how do these influence the methods chosen?

What are the limitation of these methods and how cautious need one be in interpreting the results?

Some of the problems encountered are specific to the study of aquatic Hyphomycetes whilst others are more general problems associated with the study of fungal ecology (see 1.1).

A summary pattern - Figure 4.1 - sets out some of these field and laboratory techniques, indicating where special problems arise.

The use and interpretation of aquatic Hyphomycete sporas has been discussed in detail in Section 2.3.6.2. Both collection of foam and filtration of water for suspended spores are quick and easy ways of surveying the spora of a river or stream. Filtration was specially developed by Iqbal and Webster (1973a) to get over Special Problem S, the biased sample of spores trapped by foam.

The study of the physiology of aquatic Hyphomycetes provides useful information on the potential of these fungi, which may help in the elucidation of their ecological role. In determining nutritional requirements or investigating environmental tolerances the usual practice is to manipulate pure cultures of the fungus to be studied (raised in the laboratory under optimum conditions as raw material for experimentation); 'Special culture conditions' are used: most of the conditions for growth are kept constant whilst others are varied to determine their effect in

isolation. For instance, growing the fungus at different temperatures, or providing it with different energy sources.

There are two main problems associated with this type of experiment: one concerns the design of the experiment - the choice of special conditions; the other involves the extrapolation of the results of such experiments to what happens under natural circumstances.

Physiological investigations use simplified and controlled environments - a pure culture of a fungus, on a defined medium at a particular temperature, for instance. These conditions have to be carefully chosen to provide the best and most appropriate 'background' environment.

For instance, Thornton was careful to investigate the best temperature, pH and aeration conditions for experiments on the growth of aquatic Hyphomycetes on different energy substrates. He wanted to make sure that observed differences in growth were due to changes in these substrates - not to limiting effects of the fungal environment.

The exploitation of a particular food source cannot be measured accurately if the 'background' medium contains a more easily exploited food source. A fungus growing on a cellulose containing medium containing sugars may exploit the sugars first and not get down to breakdown of the cellulose (Park 1973).

The behaviour of aquatic Hyphomycetes, even under well thought out experimental conditions, cannot accurately reflect their performance under field conditions. Here they occur as part of a varied microflora competing and co-operating with each other (in contrast to the pure culture in the laboratory) and all the environmental influences are acting together. For instance, laboratory experiments have shown that many fungi can use simple sugars as an energy source. However, they are unlikely to be able to use this potential in the field where they face competition from fast growing fungi which specialise in exploiting such sugars.

The results of physiological experiments seem to be much more useful in helping explain observed phenomena than in predicting behaviour.

Examination and manipulation of autumn-shed leaves, the principal natural substrate colonised and decomposed by aquatic Hyphomycetes, has provided much of the information we have on the occurrence and ecology of these fungi. This includes information on the species present, their abundance, their seasonal and successional patterns of occurrence, their activity and their decomposing ability; as well as their relative abundance, activity and decomposing ability compared with other members of the microflora.

Naturally occurring leaves may be taken from streams and examined for aquatic Hyphomycetes. However, many long term field programmes use 'introduced' leaves. This practice is particularly useful in the study of fungal colonisation and leaf processing since it is possible to follow the development of and seasonal and successional changes in the mycoflora, whilst monitoring the parallel progressive changes in the physical and chemical condition of the substrate.

Collecting leaves as they are shed, before immersing them in a stream means that the chemical composition and resident terrestrial mycoflora can be recorded before aquatic decomposition begins. The leaves can then be immersed in the water in such a way that they can be easily retrieved for sampling. There seem to be two main ways of doing this: Leaf discs or whole leaves, enclosed in mesh bags; or unprotected leaf-packs of whole leaves tethered together and anchored to some natural or artificial structure in the stream (e.g. Triska 1970 and Petersen and Cummins 1974).

Such a use of introduced leaves is more controlled than sampling the naturally occurring leaves and yet is not necessarily artificial. 'Natural' conditions are imitated best when whole, senescent leaves are introduced at leaf fall to an area in the stream where leaves naturally accumulate.

It is also possible to manipulate the situation: perhaps by sterilising the leaves before submerging them (Barlocher & Kendrick 1974); or protecting them from consumption by invertebrates; or by

anchoring them in an area of the stream where leaves are not usually found. Such manipulations give extra information on decomposition by aquatic Hyphomycetes and other members of the microflora.

Environment simulating experiments carried out in laboratory, and often run in parallel with field investigations, allow further manipulations to be carried out in an artificial environment which yet mirrors stream conditions. Such experiments represent a compromise between observing what actually happens in the stream (which is not an easy task - see below) and observing what happens under the totally controlled environment of a convention of physiological investigation.

Kaushik & Hynes used environment simulating experiments, submerging leaves with their natural compliment of micro-organisms in stream water containing various antibiotics to see what effect this had on the decomposition of the leaf (see Figure 3.10). Bärlocher & Kendricks parallel studies (see 3.3.2) involved the incubation of sterile leaf discs with various fungal species followed by incubation of the leaf discs in stream water at temperatures experienced at different times of the year.

To understand more about the fungi colonising submerged leaves the fungal ecologist will want to be able to see the fungi growing on the leaf, identify the species present, and get some idea of their activity and abundance. None of these tasks is straightforward and many of the problems involved have been discussed in 1.1 and 3.3. The main difficulties are shown as 'Special Problems' in Figure 4.1.

The first, 'special problem A', is the difficulty of seeing aquatic Hyphomycetes growing in or on their natural leaf substrates - since these are microfungi, intimately associated with an opaque substrate.

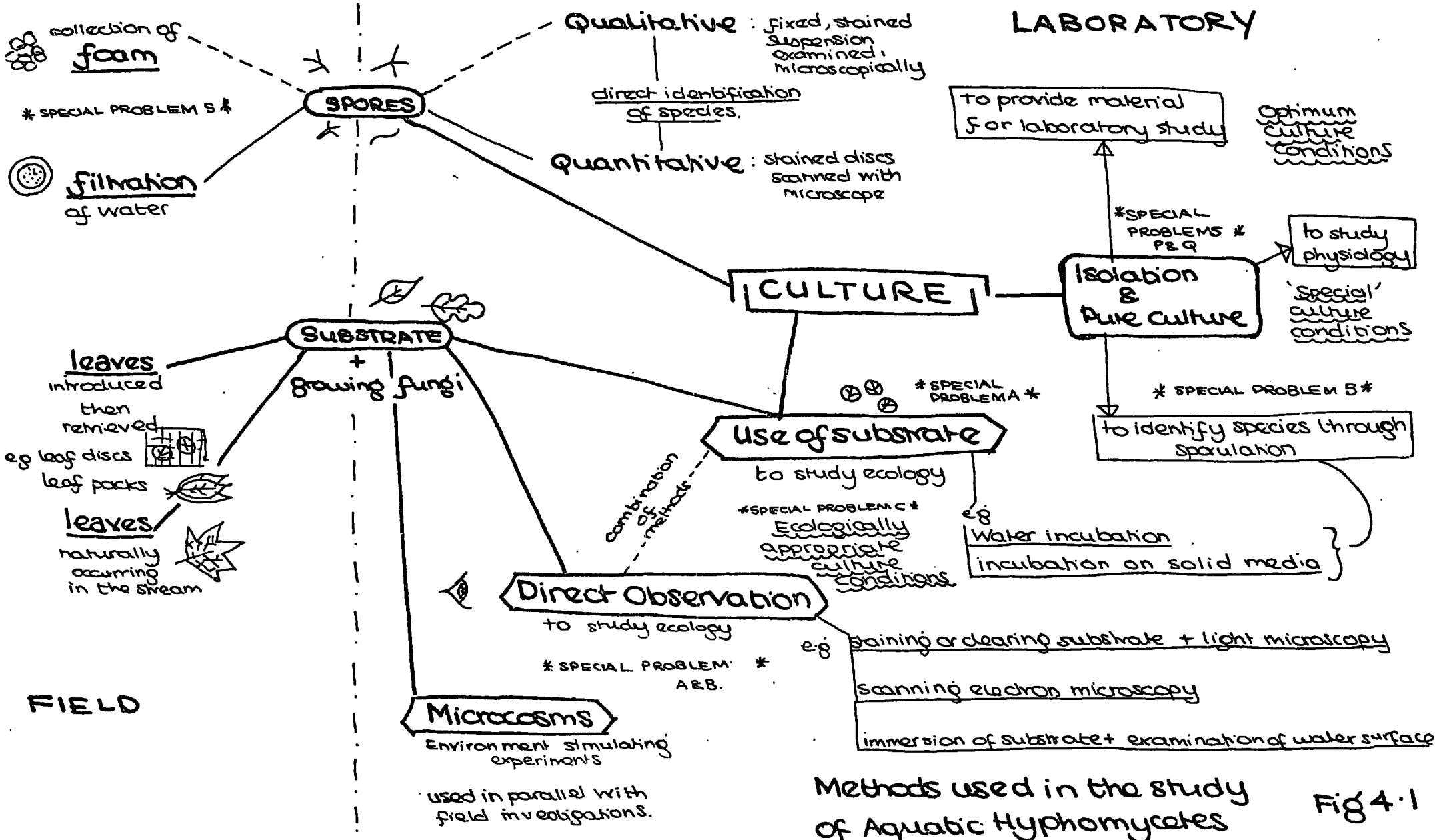
Vegetative growth of aquatic Hyphomycetes occurs within the leaf whilst the conidiophores project from the surface. Fruiting fungi may therefore be seen if the surface of the leaf is scanned using a binocular light microscope or an electron microscope. The hyphae within the leaf and the conidiophores on it will also be shown up if the leaf material is cleared and the fungi stained before microscopical examination.

Bandoni (1972) & Park (1974) also 'observed' fungi on the leaves by floating off aquatic Hyphomycete spores and identifying these. These are 'direct observation' techniques. They have the advantage of revealing what is actually happening in and on the leaf when it is retrieved from the stream (see 3.3.3).

This intimate association between fungal hyphae and the leaf also makes the measurement of fungal biomass within the leaf difficult, if not impossible. 'Indirect' methods are therefore used to estimate this ecologically important indication of fungal exploitation, for instance, the measurement of hyphal length per unit area. Or metabolism, rather than biomass, has to be measured - via respiration, or ATP content for instance.

'Special problem B' is one of identification. Aquatic Hyphomycetes in common with most other fungi, need to be fruiting before they can be identified. Direct observation reveals some sporulating fungi but not all the fungi growing actively on the leaf will necessarily be sporulating, as explained in 3.3. They may be induced to in the laboratory by incubating the leaf material.

This is where 'special problem C' occur. In examining the leaf substrate for aquatic Hyphomycetes or other fungi the ecologist is likely to have two main aims. To identify the fungi, and to get some idea of what the fungi are doing on the leaf - whether they are actively colonising and exploiting the substrate. Incubating the leaf material may solve 'special problem B' by stimulating the sporulation of active fungi and allowing them to be identified. However, unless the incubation conditions are carefully chosen such incubation may also stimulate the growth and sporulation of fungi inactive on the leaf substrate - inactive, perhaps, because the chemical composition of the leaf or the stream environment are unfavourable to growth. These fungi are not involved in the decomposition of the leaf. These problems are summarised in Table 4.1.



Methods used in the study of Aquatic Hyphomycetes

Fig 4·1

TABLE 4.1

THE ECOLOGICAL MYCOLOGIST'S DILEMMA OR THE "FUNGAL UNCERTAINTY PRINCIPLE"

MICROFUNGI

What are they?	What do they do?
identification	ecological role
Sporulation usually required:	Direct observation preferred:
therefore	but
incubation often necessary	sporulation may not have occurred
this leads to	this leads to
difficulties in assessing ECOLOGICAL ROLE	difficulties in IDENTIFICATION

Possible Solution

Combination of { Direct observation; Environmentally appropriate culturing techniques }

The solution to 'special problem C' appears to be judicious choice of 'environmentally appropriate' culture conditions, preferably combined with a direct observation technique, as in Suberkropp and Klug's study (1976). Their laboratory programme also included the use of a rich medium incubated at higher temperatures for longer periods. This addition to the range of techniques used allowed the total or potential mycoflora, active and inactive, to be revealed.

'Special problem C' makes the study of stream mycofloras particularly difficult because of the large numbers of terrestrial fungi 'present' in the stream and on the leaves. Some of these fungi are introduced with the leaf when it falls into the stream, whilst the spores of terrestrial fungi are washed into the stream from the soil to impact on the leaves. Many of these fungi grow faster and more vigorously under conventional laboratory incubation conditions than do aquatic Hyphomycetes. They become 'weed' fungi overgrowing the slow growing aquatic fungi. Once the fungi growing on a substrate have been identified and their activity established the fungal ecologist may wish to find out how vigorously each species is exploiting the substrate. Unfortunately there is not necessarily a straightforward relationship between the number of times a fungus is isolated and identified and the extent to which it is exploiting the leaf.

A rampaging fungus growing extensively and sparsely may be very frequently isolated, whilst a slow growing fungus - for instance an aquatic Hyphomycete - exploiting the substrate to the same or a greater extent, may not be isolated as frequently.

Comparisons between aquatic Hyphomycetes may be reasonably valid because they all have a relatively slow, compact growth habit and take about the same time to sporulate. Comparisons between aquatic Hyphomycetes and other fungi with different growth habits may be more difficult.

Thus the study of fungal ecology in general, and aquatic Hyphomycete ecology in particular, has all the problems that accompany the ecological study of any group of organisms and additional difficulties created by our inability to "see" what is happening, when it happens. Each method designed and employed to help us discern the true situation reveals some important feature whilst obscuring or distorting others. A judicious combination of well thought out methods is therefore essential, the limitations of each being borne in mind when the data they provide is being assessed.

4.2 A Summary of the Field Study Programme: Aims, Scope and Construction

This section explains the aims and scope of the field study programme, giving a summary of the field and laboratory procedures used, and explaining why the various methods were chosen and how the programme was constructed.

The general aim of the field study was to investigate an ecologically interesting topic; hopefully gaining insight into that topic and in the process developing expertise in the planning, execution and assessment of a programme of field and laboratory investigation.

My specific aim was to investigate the biology and ecology of a group of saprophytic aquatic fungi - the aquatic Hyphomycetes. There were several advantages to the study of this particular ecological group of fungi. Although interest in aquatic Hyphomycetes has greatly increased in recent years, the volume of literature was not too daunting. The distinctive spore morphologies meant that even an inexperienced mycologist could identify them after a short period of familiarization. Also there were several potential sites on rivers and streams within easy reach of the laboratories.

There were various limitations which restricted the aims and scope of the study. First was my lack of experience in mycological procedures in particular, and in field work in general. Fortunately the techniques chosen were relatively easy to perform; and the type of laboratory and field work envisaged required little in the way of specialized, expensive equipment.

The study had to fit the time available. Because of seasonal patterns in aquatic Hyphomycete occurrence I decided to start my investigations at the end of July. The bulk of the sampling would then be in August when the abundance and variety of spores found in flowing waters was likely to be on the increase (Iqbal and Webster 1973 b).

The study had to be carried out within rather a restricted area; the sites chosen so that they were easily and rapidly accessible (by car), and so that material could be returned without delay to the laboratory.

The choice of a 'compact' programme was therefore implied; taking

into account each of these restrictions.

A preliminary look at easily accessible sites in and around Durham City, and a first acquaintance with the more important papers (see Chapters 2 and 3) led me to adopt the programme of work summarized below.

I had several potential comparisons in mind when planning the scope of the study - involving both ecologically significant parameters as well as comparative methodology.

First, it would be possible to compare my data and observations with those of other investigators. Then interesting comparisons might be made between the data gathered from samples collected on different dates, or from different sites. Comparisons might also be possible between the species and abundance of aquatic Hyphomycetes revealed by the various 'detection' methods.

Table 4.2. shows the seven sample sites chosen. The aim was to choose several running water sites with a certain amount in common, but also with differences which might affect the aquatic Hyphomycete spora and flora observed.

All the sites - except Site G, the woodland stream - were part of the River Wear system. A look at the ordnance survey map of the environs of Durham City revealed various sites easy to reach by road and within a few miles of the laboratory. Most of these were also found to be River Authority sampling points where measurements of such ecologically interesting parameters as pH, hardness, concentration of nitrate and phosphate, and oxygen demand are made several times a year.

At all the sites the banks were wooded or the river or stream flowed through more developed woodland. Sycamore trees (Acer pseudoplatanus) overhung each site; and summer-shed leaves of sycamore could be seen in the water, especially where tumbled stones, or twigs intercepted debris.

The water at each site was fairly swift flowing and even turbulent. Only Site A showed any obvious signs of pollution and was included because of this difference.

TABLE 4.2

Sample sites

Sites - see Map 4.2		Temperature measured during study	pH measured during study	River Authority Chemical Data (1976)
A Croxdale Beck	small, shallow heavily wooded tributary of the River Wear; 2-5m wide; very cloudy water.	✓	✓	✓
B River Wear at Sunderland Bridge	main river; wooded banks; c.20m wide.	✓	✓	✓
C River Browney at A1 Bridge	small, shallow river, tributary of the River Wear; 8m wide; wooded banks upstream of sampling point.	✓	✓	✓
D River Deerness at the A690 Road Bridge	small shallow river, tributary of the River Browney; 3-6m wide; wooded banks.	✓	✓	✓
E River Browney at Stonebridge	upstream of Sites C and D; shallow, wooded banks; 4m wide.	✓	✓	✓
F River Wear at Shincliffe	main river, upstream of A-E; wooded banks; 15m wide.	✓	✓	✓
G Woodland Stream in Little High Wood	self-contained, small woodland beck; narrow (0.25-0.5m) and shallow.	✓	✓	

Two of the seven sites were on the main river. The idea here was to sample the aquatic Hyphomycete spora only; in fact collection of leaves and foam would have been difficult or impossible without a boat or other special equipment. Nilsson (N64) had suggested that the spora in such large flowing waters was likely to consist mainly of imported spores from upstream tributaries and not be the result of a well developed indigenous flora.

The tributaries themselves were sampled. At sites A and C, this was fairly close to the main river; at sites D and E, further upstream. The volume of the tributaries and other features which distinguished them from the main river and each other are given in table 4.2 and in 4.3.1.

A further contrast was expected between these relatively large flowing waters, part of a large drainage system, and the small woodland stream sampled at Site G. This stream, overhung and overshadowed by large trees, has no tributaries to import spores, but does have steep banks covered with leaf litter. The volume of runoff to the volume of stream is likely to be high.

The main programme consisted of five sampling dates in August and early September, preceded by some preliminary sampling (See Table 4.3).

On each occasion the water temperature was measured at site. Water was collected to determine its pH, and also to filter it for suspended aquatic Hyphomycete spores. Foam was collected when and where found to examine it for entrapped spores. Only at Sites D and G was foam available; though turbulent water occurred near each sample site, foam was either not produced (A, C, E, F) or could not be got at (B).

On two sample dates, leaf samples were also collected to examine them for their growing aquatic Hyphomycete floras and impacted spores. The time involved in processing such leaves meant that only the two samples were possible. Naturally occuring summer-shed sycamore leaves were collected.

TABLE 4.3

Field Sample Programme - see 4.3.2.

Date	TEMPERATURE all sites	pH all sites	WATER for filtration all sites	FOAM sites as marked	LEAVES sites as marked
28. 7.1977	-	-	(✓) G	(✓) G	-
3. 8.1977	✓	✓	(✓)	-	-
10. 8.1977	✓	✓	✓	✓ D, G	(✓)
18. 8.1977	✓	✓	✓	✓ G	-
24. 8.1977	✓	✓	✓	✓ D	✓ ACDEG
1. 9.1977	✓	✓	✓	✓ D	-
8. 9.1977	✓	✓	✓	✓ D, G	✓ ACDEG

(✓) = preliminary sample

✓ = main sample programme

The investigation used a combination of methods in the detection of aquatic Hyphomycetes. This was to allow a composite picture of the flora and spora of each site to be built up by pooling the data from the various techniques. The different pictures revealed by each method could also be compared. See Table 4.4.

The spora of each site was surveyed quantitatively by filtration of the water for suspended spores (Iqbal and Webster 1973 b), and qualitatively by examination of foam (e.g. Ingold 1974). Naturally occurring leaves from sites A, C, D, E and G were examined before and after incubation for the diversity and abundance of aquatic Hyphomycetes growing on their typical substrate, as well as for impacted spores.

The combination of leaf observation techniques was a modest version of Suberkropp and Klugs' comparative programme (1976), being chosen for the same sound ecological and mycological reasons (see 3.3.3 and 4.1).

Leaf discs were cut from the collected leaves. Some were fixed immediately so that subsequent microscopic examination would show what species had been sporulating in situ. Water incubation of leaf discs in filter-sterilized stream water and on the ISA solid plates were both carried out at 12°C. The range of water temperatures measured on 3.8.1977 was 11.5°C to 13.5°C. To make the "environmental simulation" more precise the leaf discs had to be incubated at an appropriate stream temperature. Of the constant temperature rooms available, 12°C was the most suitable, falling within the measured range. The water temperatures did drop during the sampling period but all incubations were carried out at the same temperature.

After 3 days incubation leaf discs were removed from the water or medium and examined (after fixing and staining) for aquatic Hyphomycetes. The aim was to compare the species revealed at day 0 (direct observation) and after 3 days; and combine the data from these two to get a picture of the active flora. (See 3.3.3. and 4.1).

TABLE 4.4

Laboratory Programme - see 4.3.3. and 4.3.4.

MATERIALS	PREPARATION			OBSERVATION
FOAM qualitative spore samples	allowed to settle to produce "foam liquid"		fixed and stained	drop under coverslip scanned at x 150 under binocular microscope
FILTER quantitative spore samples	water filtered through "Millipore" filter disc		fixed and stained	disc debris - side up under coverslip; scanned at x 150 under binocular microscope
LEAVES impacted spores and growing fungi	Direct observation leaves washed; discs cut		leaf discs immediately fixed and stained	Both sides of each disc scanned at x 150 using special microscope slide (Fig. 4.10)
	Water incubation leaves washed; discs cut	leaf discs incubated in filter sterilized stream water, at 12°C for 3,7 and 10 days	leaf discs removed from water, fixed and stained	
	ISA (solid medium) incubation leaves washed; discs cut	leaf discs incubated on ISA at 12°C for 3, 7 and 10 days	leaf discs removed from surface of medium, fixed and stained	plates also scanned (unstained) for growing fungi and spores

Incubations were also extended to 7 and 10 days. This allowed inactive fungi to start growing and show themselves and I was interesred to see how much of a contrast there would be between those longer incubations and the flora revealed at day 0 and day 3. A difference was also possible between the water and ISA incubations.

Suberkropp and Klugs' density score was used (see table 4.5) to give more information on the fungi observed on each leaf disc.

A more detailed description of the procedures involved in the field and the laboratory is given in the next section.

Looking back at my original plans and aims when the field study had been completed I realised that I had constructed a programme that could have adequately provided a three-year doctorate study.

In other words, the experience of carrying out the programme made it clear that the scope was a little too wide. Since the study proposed so many possible comparisons the data obtained was rather spread out. The effect of this on interpretation of the results is discussed in 5.2.2

When I started to carry out the field sample programme I was not aware just how much thought and effort was required to co-ordinate a large number of relatively uncomplicated laboratory and field procedures - to prevent unnecessary waste of time, and damage to material.

Preliminary surveys of the site had been made and I had also spent a great deal of time familiarizing myself with the rich spora that could be collected in foam from Site G - conveniently close to the laboratory.

Various practical difficulties arose during the first attempt at a complete sample and the data from these had to be abandoned and modifications made to the subsequent sampling procedure.

Not all the difficulties could be eliminated. Such a short term study is equivalent to the preliminary survey which precedes a long term study. In such a preliminary survey most of the potential difficulties and drawbacks, biological and practical, are discovered and either ironed-out or catered for.

TABLE 4.5

DENSITY SCORES FOR AQUATIC HYPHOMYCETES (after Suberkropp & Klug, 1976)

An indication of the density of each fungus on each leaf disc (direct observation, water incubation, ISA incubation).

0	only loose spores present
1	low density: observed on only one portion of the disc
2	medium to high density: observed in several areas of the disc
3	very high density, observed on the entire surface of the disc

In a study such as this there is no moving-on to a more sophisticated and informed programme of work; the data obtained have to be interpreted with any impediments firmly in mind.

This need not detract from the value of the work, however, at least to the student carrying out the investigation - since the experience gained in choosing, using and interpreting various techniques is invaluable, and gives additional insight into the comprehensive and flexible outlook that must be developed in dealing with the biological and practical aspects of an ecological study.

4.3 Methods and Materials

4.3.1 Sample Sites

Seven sample sites were chosen: A, B, C, D, E, F and G. These are marked on Maps 4.1 and 4.2. All these sites except the woodland stream, site G, are also River Authority sample points: they were easy to get to from main roads and certain chemical data were available for them.

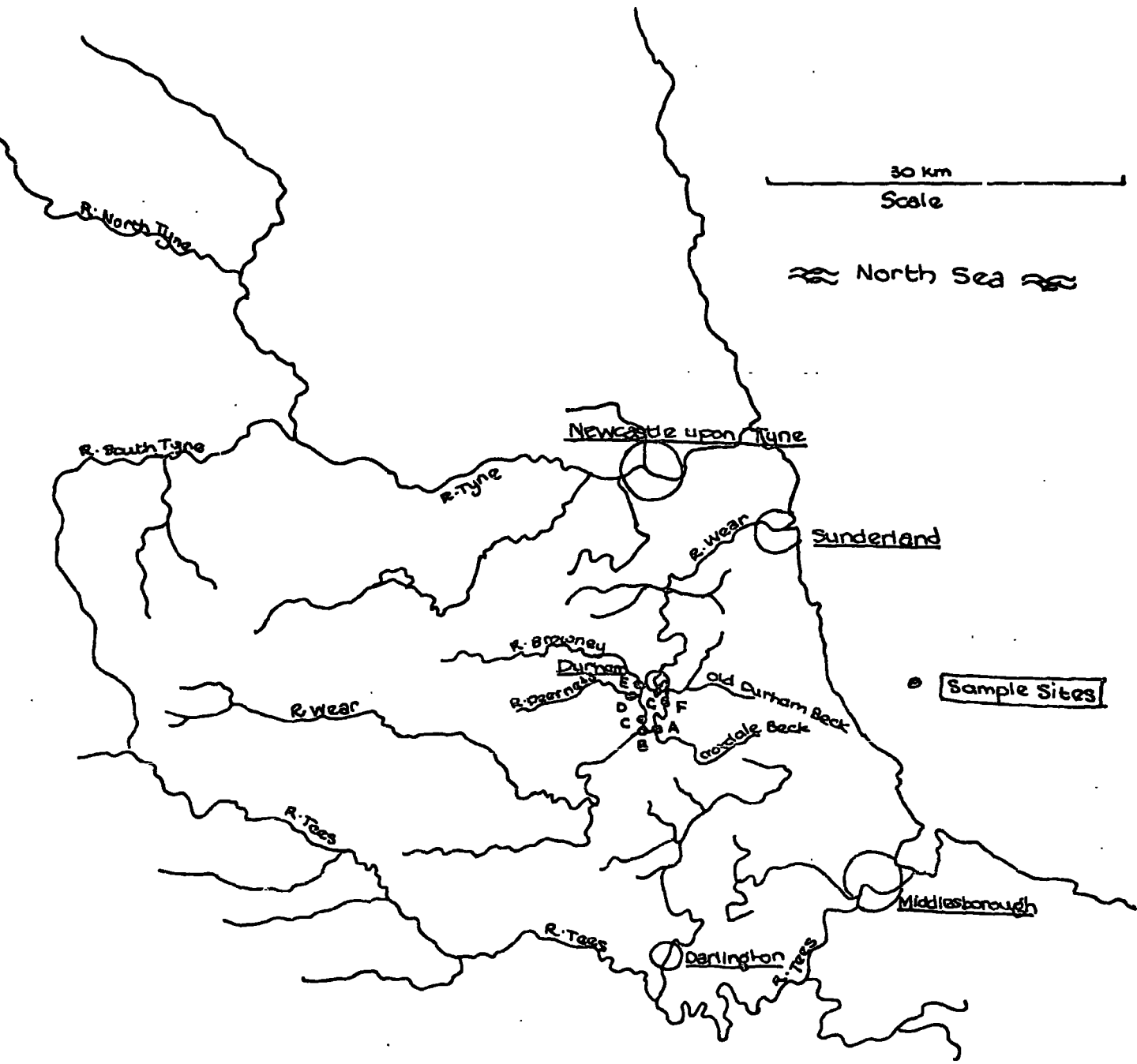
All these sites were within easy reach of both the centre of Durham city and each other. This made it possible to sample all sites in a single morning, return the samples to the laboratory within one or two hours of collection, and start laboratory procedures at once.

All the streams and rivers on which sites were chosen were bordered with trees or woodland. They differed considerably at the sample sites, in volume - width and depth - and also in details of clarity, flow, bed and bank. Sites B and F were on the main river itself. Site A was on a small, shallow heavily wooded tributary. Site C was on a large tributary close to where it joined the main river; whilst sites D and E were on main tributaries of the Wear some distance from the main river and both upstream of C. Site G was on a small stream in a steep, wooded area bordering the University science site.

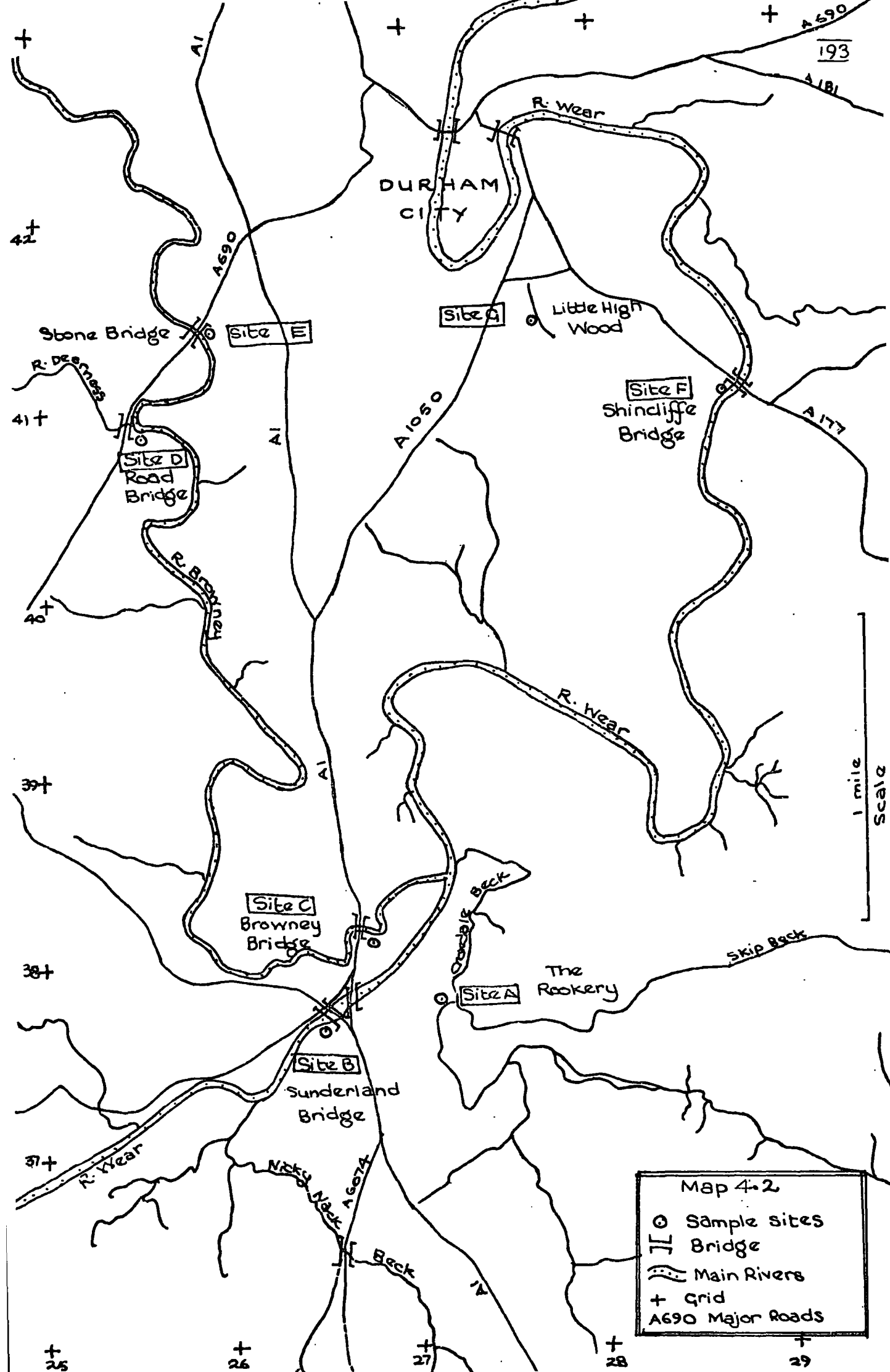
The following descriptions of the seven sites were appropriate at the time of sampling. However, width, depth, flow and clarity of the water probably alter with rainfall and time of year.

SITE A: Croxdale Beck (The Rookery) NZ 272 378

Croxdale Beck is a tributary of the River Wear, joining the main river about 3 km from the sample site. Its banks above and below the site are bordering with grazed parkland and woodland.


30 km
Scale
North Sea
R. North Tyne
R. South Tyne
R. Tyne
Newcastle upon Tyne
Sunderland
R. Wear
R. Browney
Durham
R. Deerness
R. Wear
Old Durham Beck
Sample Sites
A
B
C
D
E
F
Gordale Beck
R. Tees
R. Tees
Darlington
Tees
Middlesborough

Map 41


A1
A690
A181
R. Wear
DURHAM CITY
42
A690
Stone Bridge
Site E
Site G
Little High Wood
R. Deerness
41
A1
A1050
Site F
Shincliffe Bridge
A177
Site D
Road Bridge
R. Browney
40
R. Wear
1 mile
Scale
39
A1
Site C
Browney Bridge
Cocken Beck
38
Skip Beck
The Rookery
Site A
Site B
Sunderland Bridge
37
R. Wear
Nicky Nack Beck
A1074
A1
25
26
27
28
29
Map 4.2
Sample sites
Bridge
Main Rivers
Grid
A690 Major Roads

In the vicinity of the sample site the Beck is 2 to 5 metres wide and 5 to 20 cm. deep. The bank, 0.5 to 1.5m. high, is mostly earth, reinforced artificially with stone near "The Rookery's" access bridge. Cattle, which frequently visit the water to drink have caused much erosion of the earth banks. The bed is stony: gravel with large blocks of stone which interrupt the flow in this shallow brisk area (see fig. 4.3).

The water appears grey-brown from suspended, visible particles which cloud the water. These are probably stirred up by the cattle. The stones, and the leaves and other debris caught against them, are coated in slime where these particles have impacted.

Predominant trees are wych elm (Ulmusglabra), ash (Fraxinus) and sycamore (Acer pseudoplatanus), with shrubs of rose and hawthorn (Crataegus).

SITE B: The River Wear at Sunderland Bridge NZ 265 377

The sample site is a reinforced part of the bank right next to, and upstream of, Sunderland Bridge.

At this point the river is at least 20m wide; it is 40 cm. deep at the bank but apparently much deeper further out. The banks are wooded, or grassed with scattered trees and shrubs on a long slope leading down to the water's edge. The bed near the bank is silt, sand and small stones; larger stones, with attached 'streamers' of algae, occur further out (see Figs 4.4 and 4.5).

The water is clear with a slow, smooth flow near the bank.

Predominant trees are Scots pine, (Pinus sylvestris), ash (Fraxinus), sycamore (Acer Psuedoplatanus), willow (Salix sp.) and beech (Fagus), with hawthorn shrubs (Crataegus).

Fig 4.2

CROXDALE BECK

SITE A

Showing the shallow, rapid flow over stones at the sampling point.

Fig.4.3

CROXDALE BECK

Upstream of Site A

Showing overhanging sycamore trees, drinking cattle, and the cloudy water.

Fig 4.4

RIVER WEAR AT SUNDERLAND BRIDGE

Just downstream of the Sunderland Bridge and Site B; showing algae and wooded banks.

Fig 4.5

RIVER WEAR AT SUNDERLAND BRIDGE

Just upstream of Site B; showing wooded banks, some marginal vegetation and algae.

SITE C: The River Browney at the A.1. Bridge NZ 267 382

The River Browney is a small river which joins the Wear about 1.5 km. downstream from the bridge.

At the sample site the river is about 8m. wide and 5 to 30 cm deep. The banks, which are about 2m. high, are completely covered with a tall species of Impatiens known locally as 'Policemans' Helmets'. This grows up to 2m. high (see Fig. 4.6). Just upstream the banks are steep and wooded.

The bed is very level - fine gravel and silt with algal 'streamers'. Large stones occur in groups under the bridge, trapping leaves and other debris. The flow is smooth and fast, and the water usually clear.

The predominant trees are ash (Fraxinus), and sycamore (Acer pseudoplatanus) with hawthorn shrubs (Crataegus). These hang over the river upstream from the site.

SITE D: River Deerness at Road Bridge, A690 NZ 255 409

The Deerness is a small river, a tributary of the Browney which it joins some 100 m. downstream of the bridge.

The river is 3 to 6m wide at the sampling point. The depth is extremely variable, anything from 5 to 60 cm., as is the flow as the water flows amonst large stones 'tumbled' on the sandy bed causing miniature rapids and whirlpools. The fast-flowing, clear water creates foam at this point, and leaves and other debris accumulate against the upstream side of the stones. Just downstream of the site the bed is smoother, with a sandy 'shore' on one side. The high earth bank on this side is 1 to 2m. high, opposite a herb covered steep bank with overhanging trees (see Fig. 4.7).

The water is clear with encrusting algae, algal streamers, and Enteromorpha.

Predominant trees are horse chestnut (Aesculus hippocastanum), willow (Salix) and sycamore (Acer psuedoplatanus).

Fig 4.5

THE RIVER BROWNEY AT THE A.1 BRIDGE

Just downstream of Site C; showing dense stands of Impatiens sp. obscuring the bank.

Fig 4.7

THE RIVER DEERNESS AT THE A690 ROAD BRIDGE

Just downstream of Site D; showing wooded and herbaceous banks, stony 'shore' and the varied turbulent and smooth flow of the water.

SITE E:River Browney at Stonebridge NZ 258 414

Here the River Browney is upstream of Site C and quite different in character.

At the sampling point the river is about 4m wide. In flat areas the depth is 10-12 cm. but there are also areas of large tumbled rocks and stones which cause much variation in depth and interrupt the flow, which is generally rather rapid. These large stones trap leaves and other debris. The smooth part of the bed consists of almost horizontal flat rocks with 'steps' at intervals of a few metres. The earth banks are almost vertical and up to 4m. high with herbs and many overhanging shrubs and trees (see fig. 4.8).

The water appears clear but sunlight reveals large suspended particles which accumulate on the algal streamers attached to the flat rocks.

Predominant trees are willow (Salix), sycamore (Acer pseudoplatanus), ash (Fraxinus) and hawthorn (Crataegus).

SITE F:The River Wear at Shincliffe NZ 288 411

Site F is downstream of Site B and the confluence of the Browney.

At the sampling point, just downstream of the Shincliffe Bridge, the river is about 15m. wide and 50 cm. deep at the bank, although obviously much deeper towards the middle. The flow is smooth and not particualrly rapid near the bank but powerful further out.

The bed is silt, sand and pebbles. The water is clear but the algal streamers present accumulate quantities of silt.

Both banks slope steeply down to the water and are up to 4 metres high. They are covered in shrubs and trees, or are grass backed by trees and shrubs.

The predominant trees are ash (Fraxinus), beech (Fagus), horse chestnut (Aesculus), sycamore (Acer psuedoplatanus), and willow (Salix) with hawthorn shrubs (Crataegus).

Fig 4.8

THE RIVER BROWNEY AT STONEBRIDGE

Just downstream of Site E, viewed from the bridge; showing overhanging trees, steep banks, and stone 'steps' in the bed.

Fig 4.9

THE RIVER WEAR AT SHINCLIFFE BRIDGE

At Site F; showing earth, wooded and grassed banks. Upstream can be seen a narrowing of the bank and rapids over stones.

SITE G:Woodland Stream in Little High Wood NZ 277 415

This site is on a narrow woodland brook which flows down a steep wooded slope in the Little High Wood.

This brook appears from beneath a dense vegetation - including bracken, Equisetum, and grass - flows down the slope for about a hundred metres and then disappears underground at the wood margin, beneath the science laboratories site.

The brook is narrow along the whole length of flow, being about 0.25 to 0.5 m. wide at the sampling point. The banks are very shallow and obscured by vegetation at the point where it emerges. However, at the sampling point further down the slope, the banks are 3 to 4m high with mature trees, shrubs and mixed herbaceous vegetation.

The bed is mud, lined with leaves. These were mainly beech at the time of sampling. The course of the brook is rather irregular with 'steps' and small pools created by twigs and branches wedged across the flow. The depth, therefore, is very irregular (2 to 10 cm. at the sampling point ; as is the flow which varies much more obviously with the weather than at the other sites. Foam and scum accumulate against the obstructions, but the water is clear with no evidence of algal growth.

There is an abundance of overhanging trees which means the water is heavily shaded at all times of day. Beech (Fagus), sweet chestnut (Castanea), oak (Quercus) and sycamore (Acer pseudoplatanus) are predominant; elder (Sambacus) and hazel (Corylus) also being present.

4.3.2 Field methods

Table 4.3 summarises the samples taken and measurement made at the various sample sites on dates in August and September 1977. On each occasion and at each of the sites the temperature of the water was measured; water was collected to determine the pH and to filter for suspended fungal spores; and foam was collected - whenever and wherever found - to examine

it for trapped spores.

On two occasions, and from five of the seven sites, submerged naturally occurring leaves were retrieved so that they could be observed - with or without incubation - for trapped spores and growing fungi.

Sampling was always carried out at the same time of day (09.00 to 12.00) and the sit es were visited in the same order on each occasion. Processing of samples was carried out in this same order to ensure that the material from each site was in a similar condition when dealt with.

On reaching each sample point a thermometer was totally immersed in the water for some minutes before the temperature of the water was recorded.

Water needed for determination of pH and for filtration was collected in clean, screw-top polythene bottles of 1 litre capacity. These were well rinsed out with the river or stream water to be sampled before being filled. Care was taken not to scoop up bottom deposits.

Where foam occurred it was carefully scooped up with a spoon, pouring off the excess water, and left to stand in a small watertight polythene container.

Leaves were collected from the upstream side of large stones and twigs which trap leaves and other debris as they are carried downstream. These leaves were put into new plastic bags.

Leaves were never abundant at any site except Site G where large quantities of beech, a rather resistant leaf, line the bed of the stream. However, summer-shed leaves of sycamore (Acer Psuedoplatanus) could be found at all the sites and so these were collected in preference to other species. Only on one occasion was this not possible, at Site C: willow leaves were collected instead. Leaves showing obvious thinning

and skeletonisation were chosen if available. It was thought that these might reveal a more diverse and abundant mycoflora than intact, recently shed leaves. Certainly thin and skeletonised material is far easier to scan under the microscope; more light passes through and the projecting sporophores of aquatic hyphomycetes are easier to see. However, since few leaves were available, not all of those collected were in this 'ideal' condition.

4.3.3 Laboratory methods: preparation of materials.

During sampling all materials were kept as cool as possible and on return to the laboratory were placed in a 9°C cold room until they could be processed.

Water samples: pH measurements were made immediately on return to the laboratory. The water collection bottles were thoroughly shaken before about 200 ml. was withdrawn and the reading taken.

Filtration of the water was then carried out to remove all suspended spores from a known volume of water. The equipment available was a 2.5 cm. diameter "Millipore" filter apparatus with a 15 ml. funnel. This was clamped to a one litre capacity Buchner flask; the suction being created by a fast running tap. The filter discs used were also "Millipore" with an 8 µm pore size (Type SC). This large pore size allowed a fairly rapid filtration of the water sample whilst retaining particles of the appropriate size range on the surface of the filter disc. (Ingold & Webster 197 b).

Usually two filter discs, sometimes three, were prepared from each site. Because of the variable amounts of suspended material present in the water samples - particles of dirt and algal cells for instance - the volume that could be filtered through a simple disc varied from site to

site and on different dates. The retention of too thick a layer of fine debris slowed down filtering considerably and made observation of the stained disc very difficult.

For instance: the cloudy water collected from Site A very rapidly clogged the pores of the filter and built up to a thick dark brown layer on the surface. The volume that could be passed through filter without totally obscuring it was therefore very low, only 25 or 50 ml., and an extra filter was taken.

At Site B on the River Wear the density of algal cells was consistently high: large numbers of these cells built up as a green layer on the filter surface, obscuring any spores present and restricting the amount of light that could pass through the stained filter. Low volumes of water were therefore passed through each disc, usually 50 ml.

Conversely, at Site C the amount of suspended material was rather low; the number of spores retrieved was also low therefore a relatively large volume was passed through each filter, usually 200 ml.

At Site G the density of spores was often rather high and the volume filtered per disc had to be reduced so that the spores could be seen and counted easily.

After filtration was complete, the filter discs were carefully removed from the apparatus so that no harm was done to the surface layer of material. They were placed in glass petri-dishes and carefully flooded with a dilute solution of lactophenol cotton blue - a dual purpose preservative and stain colution. Care was taken not to dislodge any of the material embedded in the surface of the disc. The lactophenol cotton blue not only fixed the trapped spores but made them easier to see by staining them blue, and by rendering the background filter disc more transparent. After 24 hours staining was sufficient, but the discs could be kept moist in the petri-dishes for some time without deterioration.

However, dried out discs were almost impossible to see through, so the liquid in the petri dishes was never allowed to evaporate completely. The easiest disc observations were made with damp discs, neither dried out nor flooded with staining solution. Lactophenol cotton blue stained all the living material caught on the filters as well as some of the non-living particles. Aquatic hyphomycetes spores are of such distinctive shape that they were easy to pick out amongst a mass of algal cells or other debris, as long as these did not actually obscure them.

Foam samples: these were left to stand until all the bubbles had subsided (one to several hours). The resulting liquid, usually about 10 ml., was poured into a snap-top specimen tube where it was fixed with an equal volume of formo-acetic alcohol. A few drops of lacto-phenol cotton blue were added to stain the spores; this staining was adequate after about 8 hours. Foam had to be looked at within 36 hours, because if it was kept longer than this the spores shrank and became deformed, making identification difficult.

Leaf Samples: Leaves from a single site were rinsed gently but thoroughly under running tap water to remove dirt, debris and animals.

Semi-sterile conditions were preserved throughout the leaf disc preparation - mainly to prevent cross contamination of samples. Each leaf was laid on a pad of clean tissue overlying a new heavy plastic bag: this stopped the leaf from slipping. Several discs were then cut from each leaf available, using a flamed cork-borer of 0.5 cm. diameter.

Since suitable material was far from abundant it was not possible to select the position of discs within the leaves randomly. Not all the leaves from the same site were of a similar condition. Usually between 3 and 10 leaves were retrieved from each site. Both green and brown leaves

were found. All were obviously summer-shed but some of these were almost wholly skeletonised whilst others had only small areas of thin or skeletonised tissue. Where the whole leaf was thin and/or skeletonised, leaf discs were cut from several areas of the leaf. However, when leaves had only small areas of suitably decayed tissue, leaf discs were cut from these. If more discs were required some of the thicker, less decayed tissue was sampled.

All the discs cut from the leaf sample from a particular site were temporarily placed in a sterile petri dish - using flamed forceps - and then sorted out so that 'mixed' samples of leaf tissue were used in various techniques. This was to help prevent apparent differences in the mycoflora revealed by the different techniques being due to a comparison of leaf materials of different condition. In some cases the leaves were so fragile that portions had to be cut from them with flamed scissors: these pieces were then treated in the same way as discs.

The discs to be used for direct observation were placed immediately in a dilute solution of lactophenol cotton blue contained in small snap-top specimen tubes. At least five discs from each site were preserved on each occasion.

Two types of incubation of leaf material were used to encourage the sporulation of any aquatic hyphomycetes present and to allow them to be identified.

This was the procedure adopted for the leaf discs from each site:

In the water incubation five discs were placed in each of three sterile petri dishes half-filled with water. This was filtered stream water from the appropriate site. After the filtration of each water sample for suspended spores this once-filtered water was re-filtered using 0.22μ pore size discs. The first filtration removed most of the larger particles suspended in the water, whilst the second sterilised the water.

These plates were then placed in a 12°C constant tempeature room: one was removed after 3 days, another after 7 days and the last after 10 days. The leaf discs from each plate were transferred to specimen tubes containing the combined preservative and staining lactophenol cotton blue solution ('Full strength' stain diluted considerably with lactophenol).

For incubation on the solid medium: five discs were placed (using sterile forceps) on each of three sterile plates of ISA. These were also incubated at 12°C for 3, 7 and 10 days. Each set of five discs was removed to lactophenol cotton blue, whilst the plates were retained to observe any fungi growing out across the surface of the agar.

Discs preserved in this way from the water and ISA incubations were suitably stained for observation after about 24 hours. However, as long as they were not agitated or disturbed, these samples could be kept for some time before the discs were scanned without any damage or distortion. This allowed the scanning of various materials to be spread out over time - the foam being observed almost immediately, the filters within a couple of days, and the preserved leaf samples after that.

Plates were scanned as soon as possible but were stored in a domestic refrigerator at 4°C unless this could be done immediately.

4.3.4 Laboratory methods: observation and recording

Filter discs: these were ready for observation after about 12 hours. By this time the stain had penetrated the spores - and other living material - sufficiently for them to be seen as bright blue objects against a pale blue, semi-transparent background.

A whole filter disc, debris side up, was mounted on a conventional microscope slide and covered with a large coverslip (22 x 64 mm).

The whole area of the filter disc was scanned, using strong transmitted light, at x 150 (a combination of x 10 objective, x 10 eye-piece and a x 1.5 tube correction). Each spore found was recorded. This gave data on both the numbers and species of fungal spores from a known volume of water.

Foam samples: A drop of stained foam-liquid was removed from the bottom of the specimen tube, where debris and spores had settled out. This was mounted on a microscope slide under a 22 x 22 mm coverslip. The whole area under the coverslip was scanned using transmitted light and a x 10 objective. It was sometimes necessary to increase the power for more detailed examination of the spores.

Each aquatic hyphomycete spore found was recorded. If few spores were found in a single mounted drop, another was scanned.

These records are qualitative: they show what proportions of different species of aquatic hyphomycete spore occur in foam. This is a function of both the total spora of the site and the selective nature of spore removal by bubbles.

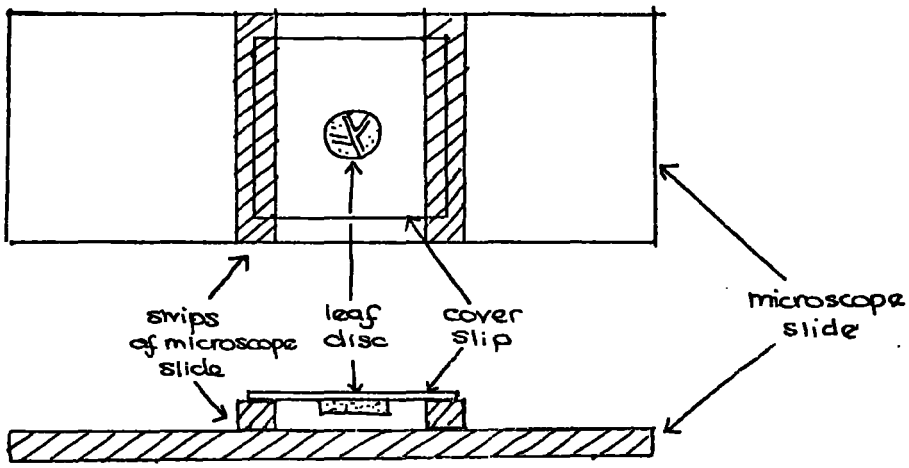
Leaf discs and plates: After sufficient staining, leaf discs were carefully removed from their preservative liquid, and placed on a 22 x 22 mm coverslip. This coverslip was then inverted over 2 parallel glass strips which had been glued about 2 cm. apart to an ordinary flat glass microscope slide (See fig. 4.10). This allowed thick specimens to be viewed without visual distortion, and without squashing the material.

The whole area of each disc was scanned at x 150, both for loose spores and those attached to sporophores. Strong transmitted light was used.

When the leaf discs were of well-skeletonised tissue, detection and identification of spores was relatively easy. When the leaf material

Fig. 4·10

Modified microscope slide for scanning stained leaf discs.



was thicker it was sometimes necessary to scan both sides of the disc, and much stronger light had to be used. Some of the leaf tissue was still living and stained blue; this made detection especially difficult. For all discs quite a depth of field had to be scanned to make sure that no spores or sporophores were missed. The preservative liquid from each pot was also examined to ensure that spores had not been dislodged. Fortunately this was seldom a problem.

In recording the spores and sporophores observed on the surface of each disc Suberkropp & Klug's density scale was used. These give a little more information on the abundance of each species found than does a simple record of presence or absence. The scale is given in Table 4.5.

The agar surface of each incubation plate was scanned at x 150, after the leaf discs had been removed. No staining was necessary but the condenser iris was closed down considerably to create more interference and improve observation.

The data obtained from these various techniques is set out and discussed in the following chapter. Comments are also made on the limitations of the sampling programme and methods used and what effect these have on the value and interpretation of such data.

5. RESULTS, DISCUSSION AND CONCLUSIONS

5. RESULTS, DISCUSSION AND CONCLUSIONS

5.1 Introduction

My aim in this presentation of the results and conclusions from the sampling programme is not only to extract biological information from the various data collected, but also to show what insight into choice and use of various methods can be gained by devising and executing a short term practical study such as this.

My initial aim in pursuing the field study and in interpreting the results was obviously to find out what I could about the biology and ecology of aquatic Hyphomycetes in the Durham area. However, as the study progressed and the data was assessed, I found that what I could not do, and what I could not conclude - because of both practical and biological constraints (see 4.1 and 4.2) - revealed as much to me about the ecological investigation of a group of organisms, as the 'useful' data did about the aquatic Hyphomycetes that were my particular concern.

In the first main section of this chapter, 5.2., all the results from the field study are presented and commented on. First (section 5.2.1), all relevant data is set out in full (Tables 5.1 to 5.6) and also in summary (Tables 5.7, 5.8, 5.9) with general comments on what was found, and on the various difficulties that made collection of data troublesome or impossible.

Secondly, in section 5.2.2, the data is examined in more detail: possible and actual comparisons between sites, dates and methods are made (see 4.2). General comments are made in this section on the usefulness of the data. Obviously the way in which the programme was constructed (4.2 and 5.3), the abundance and condition of the

materials collected, and the expertise with the various techniques were carried out, all have a bearing on the quality of the data they helped to produce.

Section 5.2.2 is accompanied by a summary diagram, Fig. 5.2. This sets out various factors that may influence both the actual and the 'revealed' flora and spora of flowing waters. In the top half, various features of a sample site's 'environment' are set out. Such features help determine which species grow at that site, and with what success; also what spores may be retrieved from that site. The bottom half of Fig.5.2. illustrates various features of the present programme, some controllable, others not, which influence how accurately we perceive the flora and spora sampled at a particular site.

In the second main section of the Chapter, 5.3., an attempt is made to draw general conclusions about the biology and ecology of aquatic Hyphomycetes in the Durham area. The various constraints that affect the planning, execution and interpretation of a practical ecological investigation are briefly explored, with illustrations from the present study. Suggestions are made for improvements to the present programme.

5.2. Results from the Field Study

5.2.1. General comments on data and methods.

5.2.1.1. Site Data:

During the course of the field sampling programme the water temperature at each site dropped between 2 and 5°C as autumn approached (Table 5.2). The main river sites, with their larger surface area exposed to sky and sun, were almost invariably warmer than the other sites; whilst the effect of shading was particularly noticeable in the lower temperature recorded from Sites A and G.

The pH values obtained during the sampling programme (Table 5.1) were higher than the 1976 averages given by the Northumbrian Water Authority for Sites A to F. This may have been a seasonal effect or a constant discrepancy in my recorded values (although the machine used was calibrated before each set of readings). The relationship between the sites reflected in both sets of pH data was the same, however, with Site A being the most alkaline and Site B being the least alkaline of the Authority sites. The alkalinity of Croxdale Beck was also reflected in the larger amounts of calcium carbonate measured at that site (Table 5.3). The pH at Site G was remarkably constant and less alkaline than at the other sites; perhaps acid leached from the many beech leaves reduced the pH of a naturally more alkaline water.

Abundant organic particles were found suspended in Croxdale Beck water. This feature was reflected in three of the measurements given in Table 5.3 (a selection of data from the more detailed information provided by the Northumbrian Water Authority). Not only were the values for total solids much higher than those from the other sites (about 3 times as great, in fact) but the values for nitrate (nitric N) and phosphate were

also higher because of the increased organic content of the water. This site appears to be somewhat more eutrophic than the others.

A certain amount of pollution was also indicated at Site C, where nitrogen (as ammonia) and the biological oxygen demand (B.O.D.) were higher than at the other sites, and the dissolved oxygen content a little lower.

It was not possible to make comparable measurements to those given in Table 5.3 on the water from Site G. The lower pH indicates water of a rather different character.

The rainfall figures for the duration of the programme are given in Table 5.4. The dates of each field sample are indicated. The first main sample was preceded by a dry spell. All the others were preceded by a certain amount of rain; the third sample on the 24th of August following a particularly heavy fall. The effects of this heavy rain on the filters taken varied considerably (see below).

5.2.1.2. Foam and Filter Data.

Detailed data of the foam and filter sporas is set out in Table 5.5, whilst summarized data can be found in Tables 5.8 and 5.9.

Tables 5.5a → g (which can be found with the separate data and results sheet at the back) have been set out to allow easy comparison of species and numbers between dates, methods and sites.

Each site table has the same species list - the combined spora and flora from all methods throughout the programme. Although the volume of water filter and 'observed' for spores differed between sites and dates the records for Sites A to F give the total number of spores per 400 mls. Obviously this means that for sites where a small volume (50 or 100 mls) was scanned the numbers are rather artificially bulked up. If indeed, 400 mls had been scanned each date and at each site the number of species

recorded might have been slightly higher for those sites with sparse records. However the most common species recorded from such sites would probably have been the same.

The fungi listed in Table 5.5 (and 5.8) are almost exclusively aquatic Hyphomycetes. T. splendens to C. aquatica all have tetraradiate spores; F. curvula to C. pellucidum are the more common filiforms; H. lugdunensis, C. stellata and D. aquatica have less obvious projections; whilst Entomophthora onwards is a miscellaneous group of rare, unknown or non-aquatic Hyphomycete species.

C. pellucidum is the only demiataceous (dark coloured) species included. Entomophthora is included because of its distinctive tetraradiate shape. The unknowns A, B, C and D have been recorded previously whilst the K-shaped and H-shaped spores shown in Fig. 5.1 have not: these cannot be identified as either algae, or of zoological origin. They are within the same size range as aquatic Hyphomycetes and their simple branched structure suggests that they may, in fact, be aquatic Hyphomycetes.

Other spores were occasionally encountered in foam and on filters, though not with any frequency. For instance, the disinctive spores of Altenaria and Helminthosporium were occasionally observed, as were the curved spores of Fusarium. An unknown crescent-shaped ? propagule with two regularly positioned septa was also observed in several samples. These had a 3-dimensional twist to them (see Fig. 5.1). They could not be identified as fungi, algae or of zoological origin.

Other objects similar in shape and size to certain aquatic Hyphomycetes were found, and identified with the help of a algologist and a zoologist. Most striking were the clusters of crescent shaped propagules characteristic of the alga Ankistrodesmus falcatus; also the dark-staining chaetae shed by species of Naididae (rather small aquatic worms) which are highly reminiscent of the 'spurred' spores of Centrosporina acerina and

Figure 5.1.

A *Tetracladium marchalianum* spores on filter disc: x 600

B *Tetracladium setigerum* spores on filter disc: x 600

C *Articulospora tetracladia f. tetracladia* on filter disc: x 600

D *Articulospora tetracladia f. angulatum* on filter disc: x 600

E *Tricladium splendens* spore on filter disc: x 150

F *Flabellospora sp.* growing on 18A plate: x 600

G 'H' spore on filter disc: x 600

H 'K' spore on filter disc: x 600

I Unknown crescent (showing septa) on filter disc: x 600

J Unknown crescent (showing 3-D shape) on filter disc: x 600

K *Ankistrodesmus falcatus* on filter disc: x 600

L Worm chaetae on filter disc: x 600

M *Altenaria* spore on filter disc: x 600

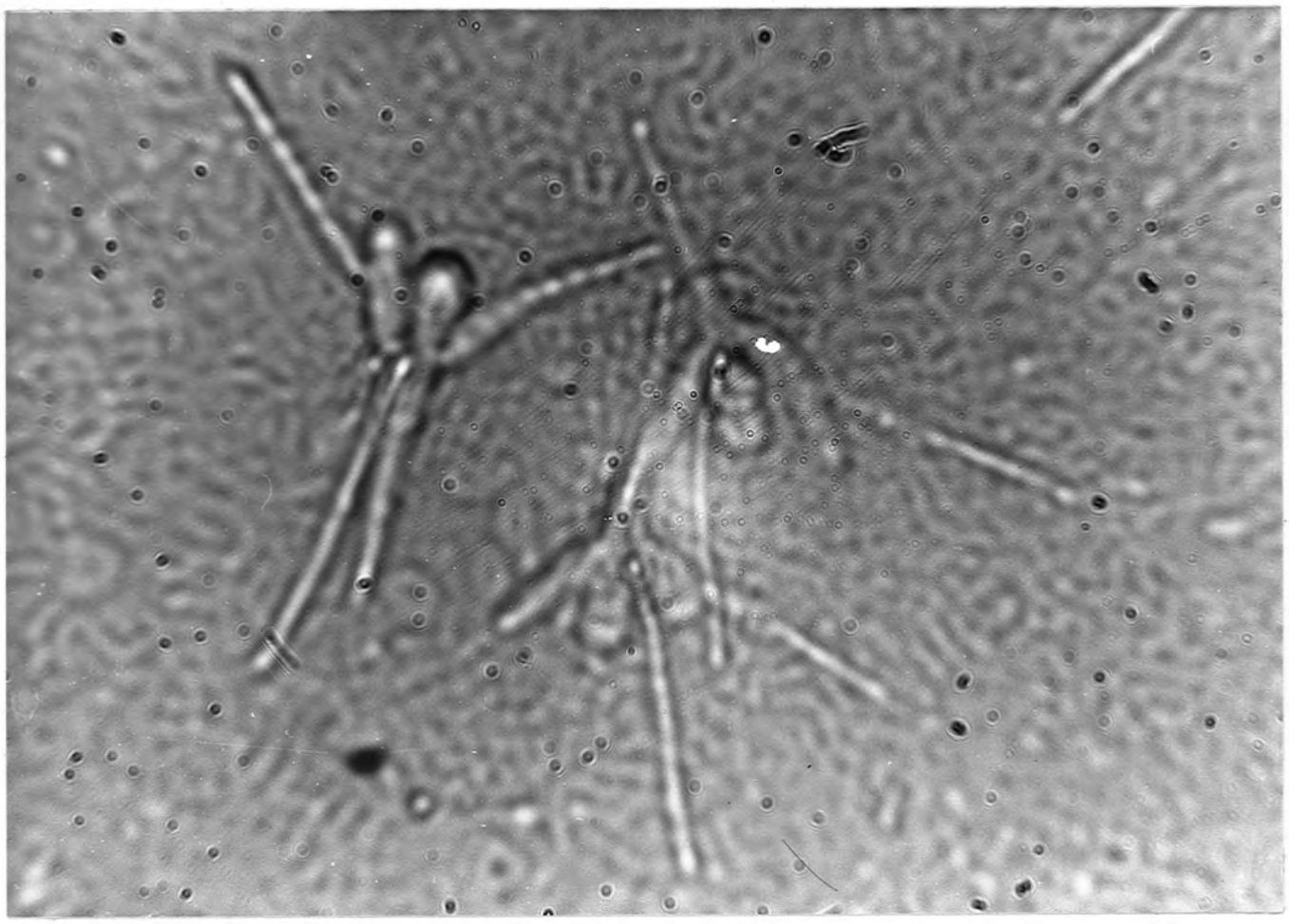
N *Entomopthora* spore on filter disc: x 600

O *Cladosporium* sporulating on plate: x 150

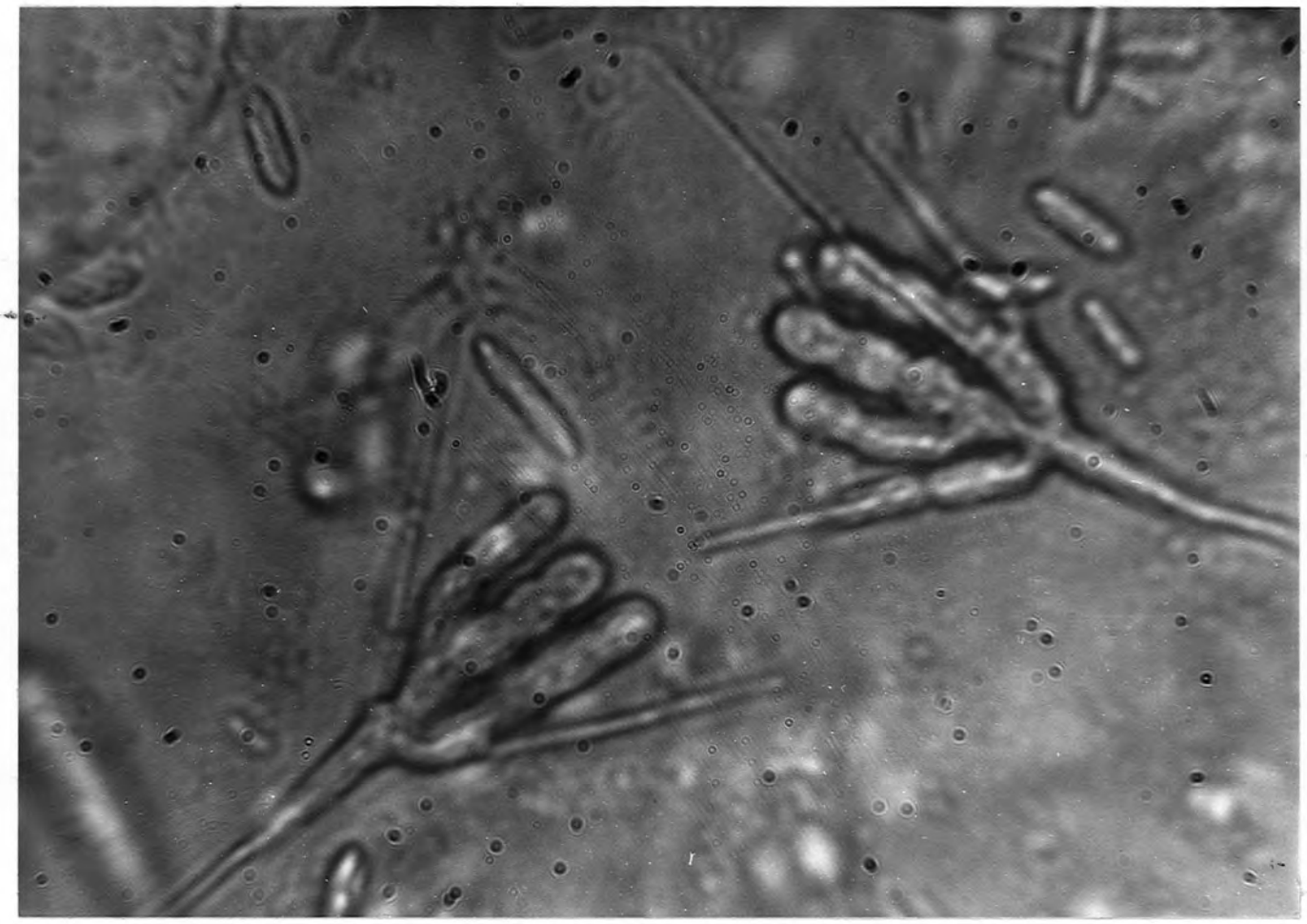
P *Mucor*: collapsed sporophores on plate: x 150

Q *Fusarium*: group of spores on plate: x 150

R *Aureobasidium*: sporulating on plate: x 150



A



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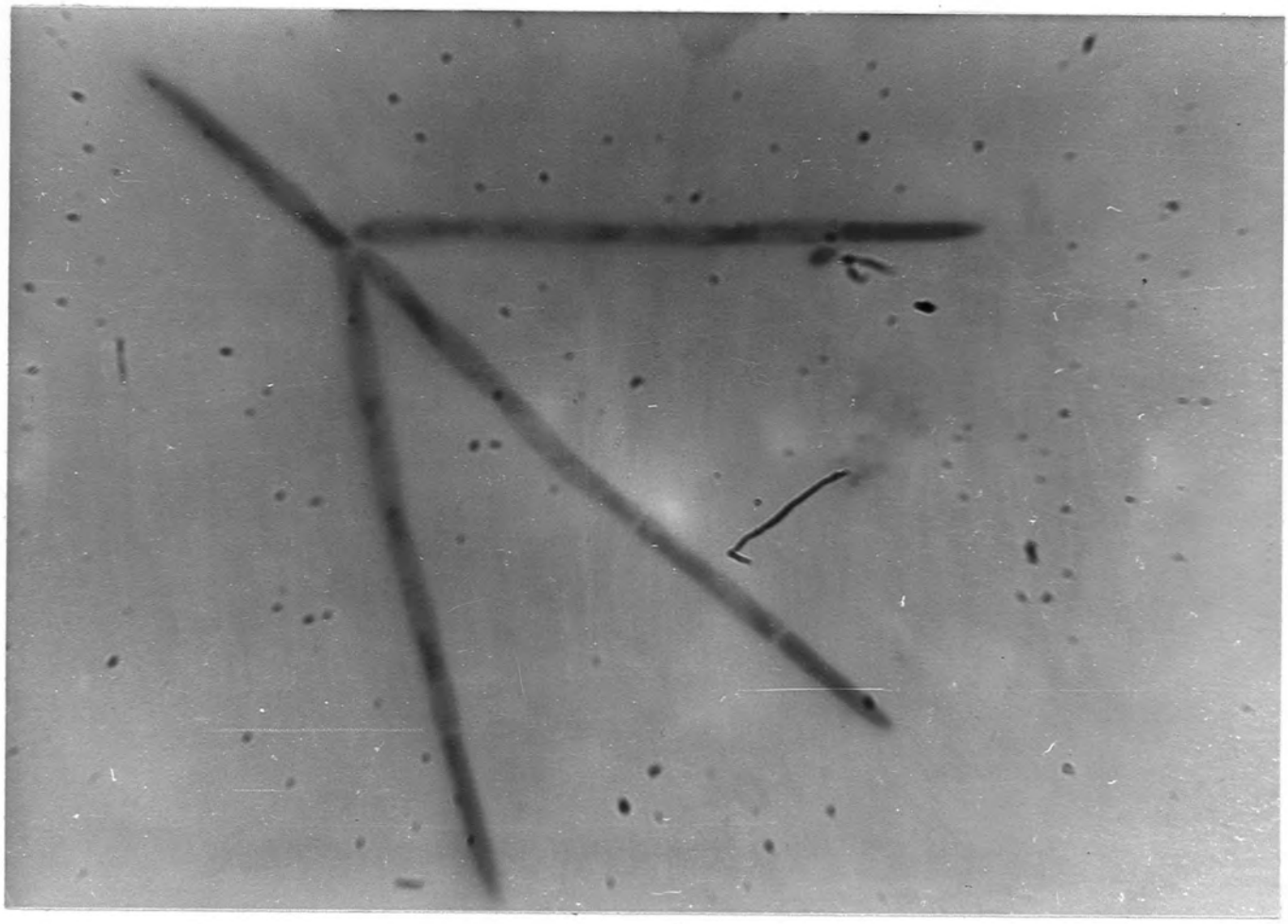

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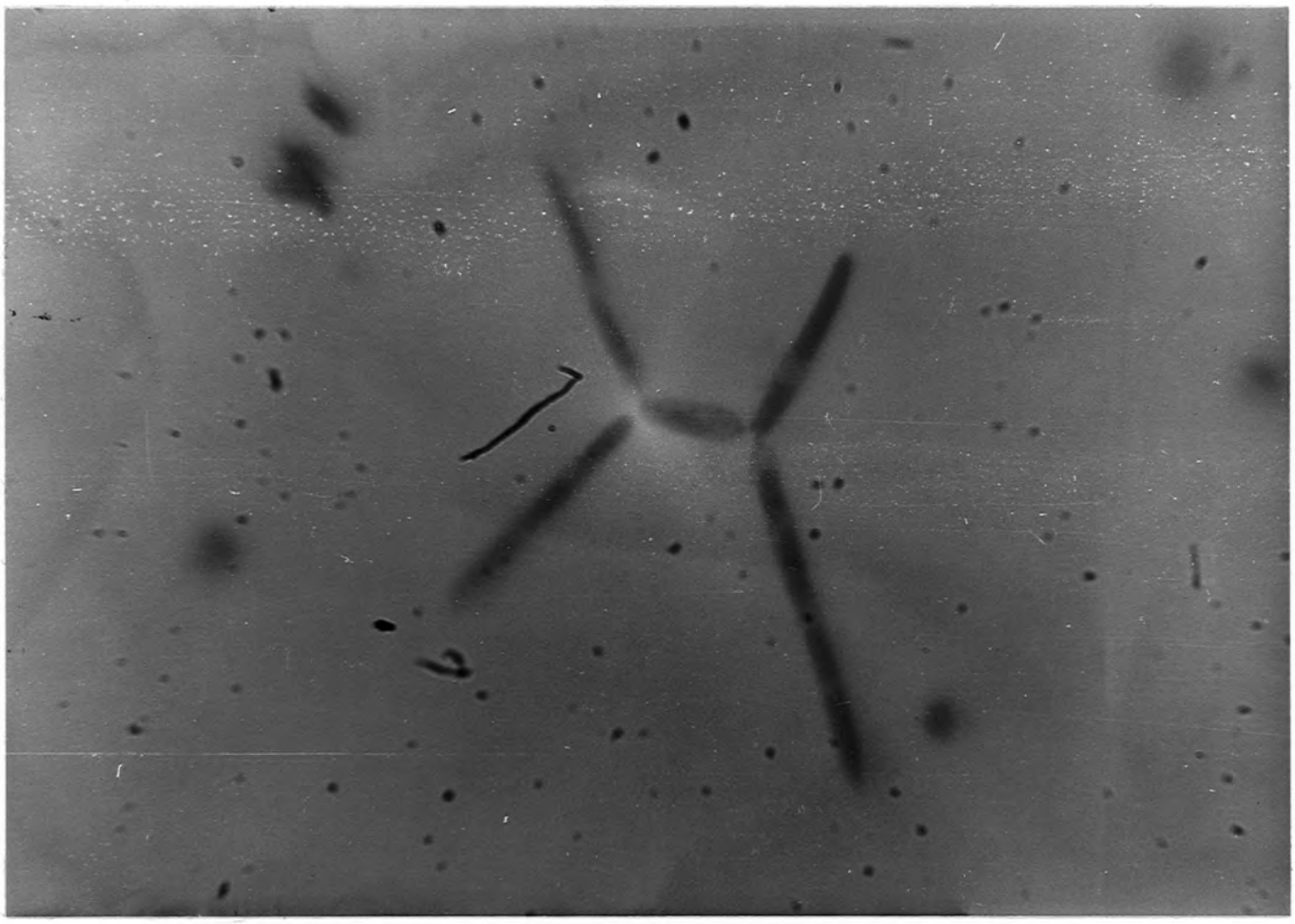

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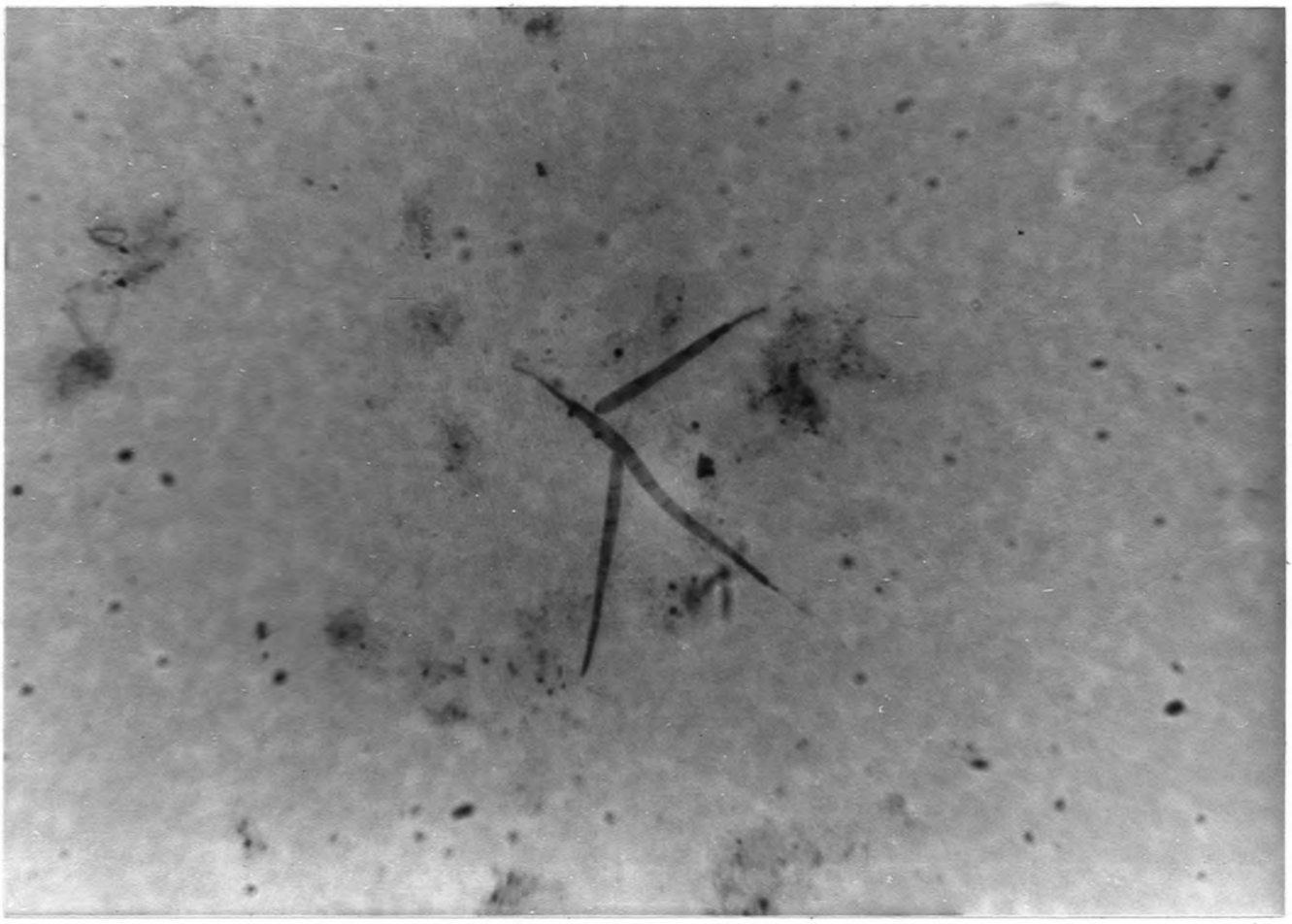

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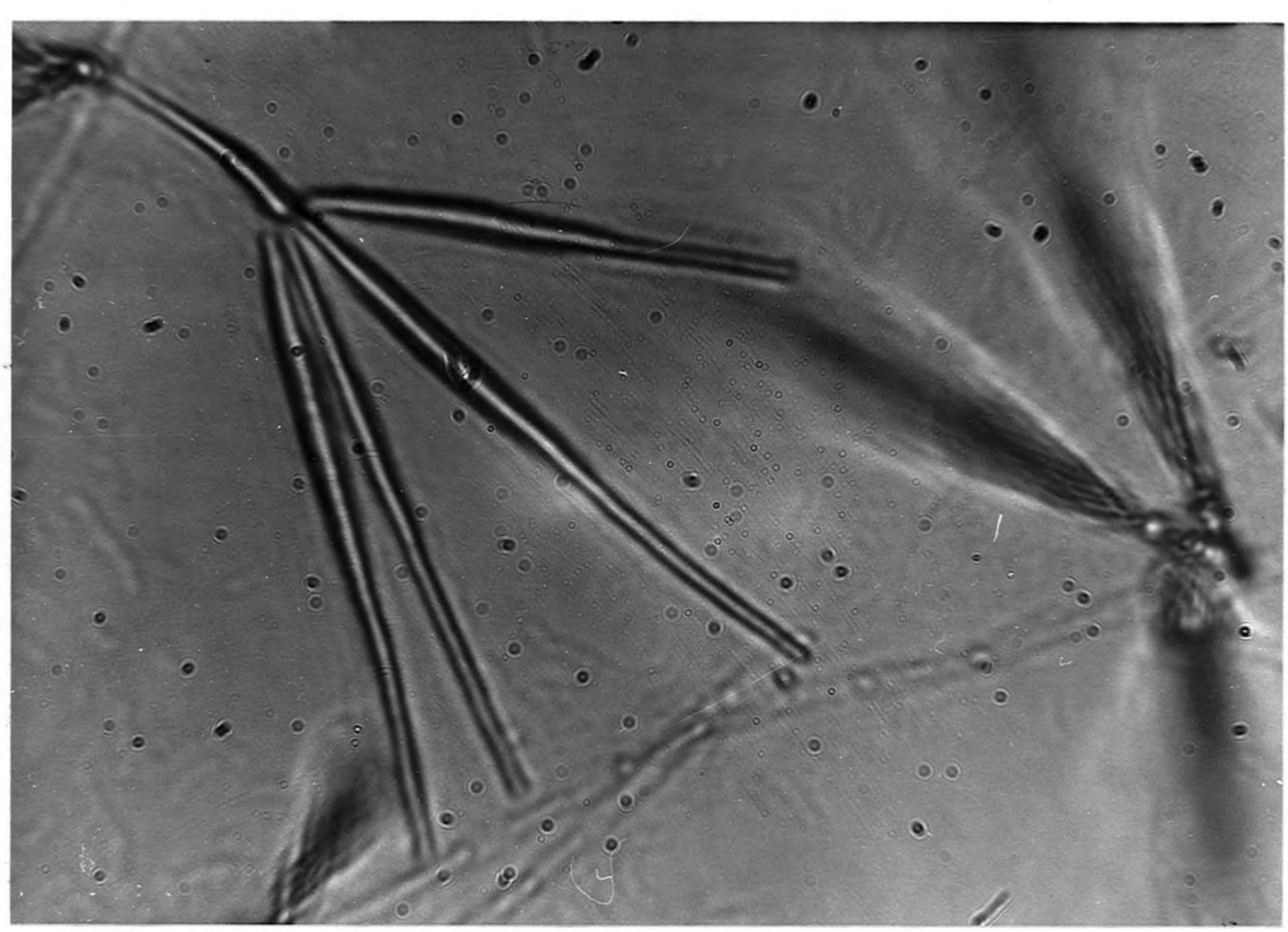

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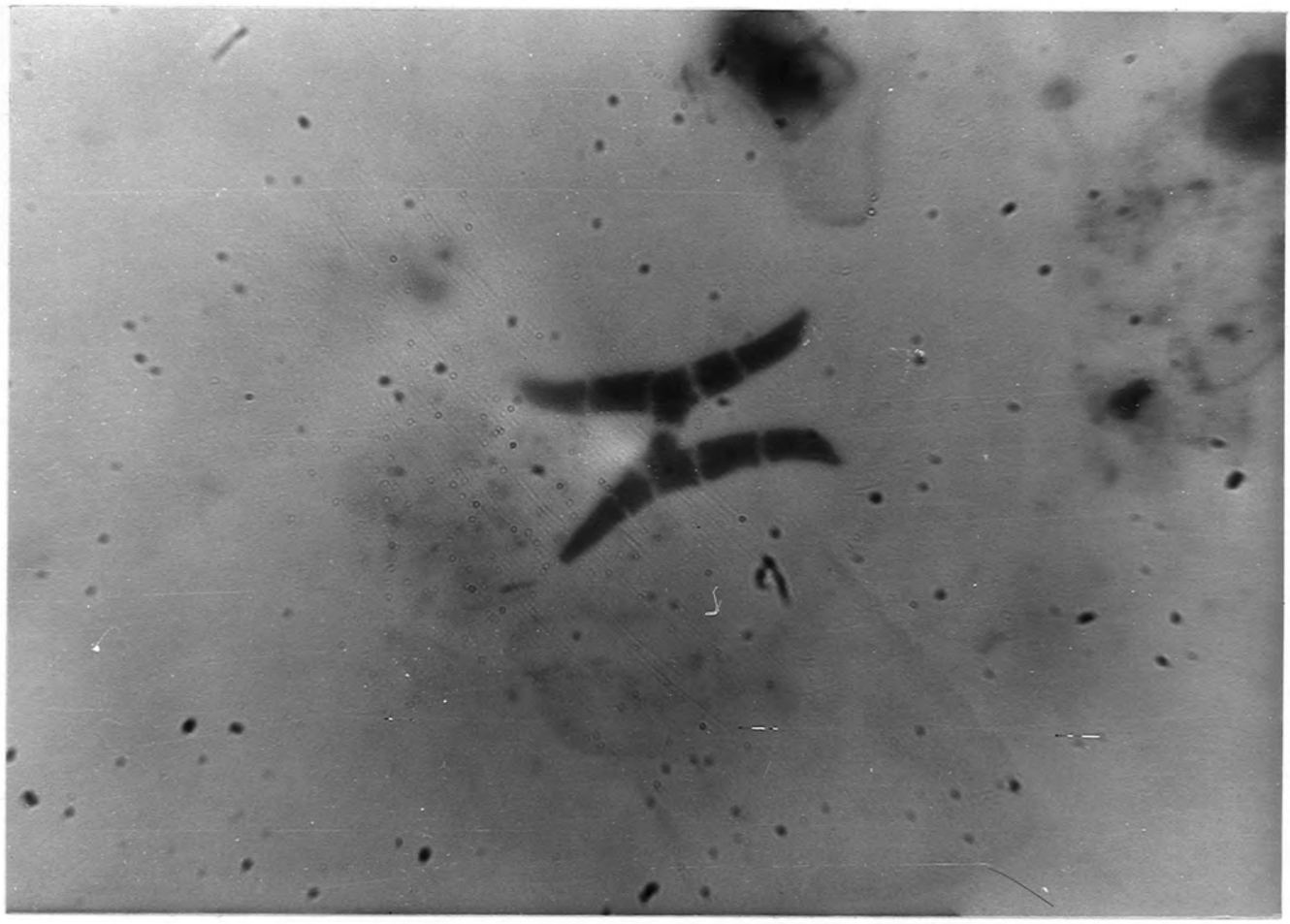

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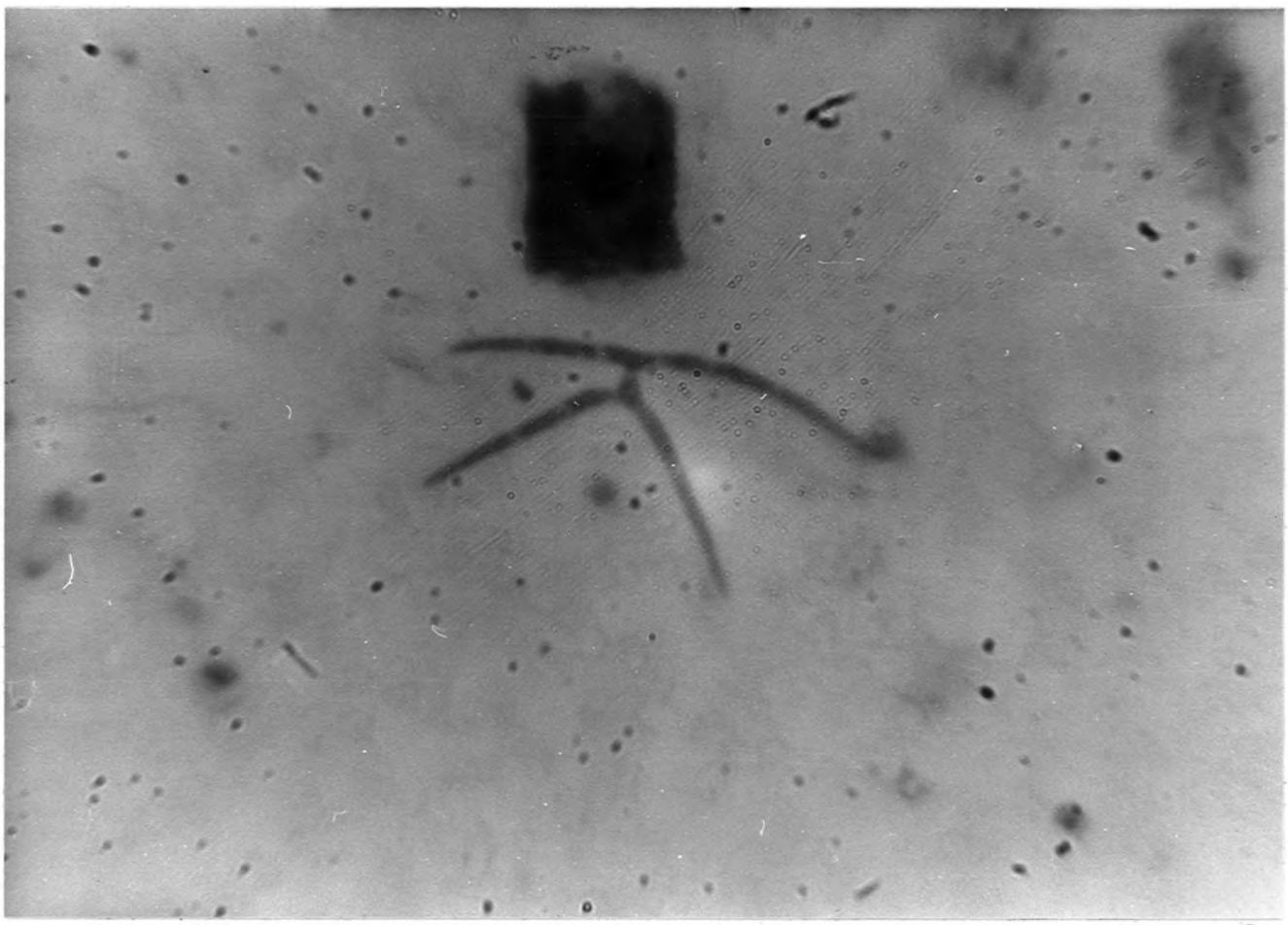

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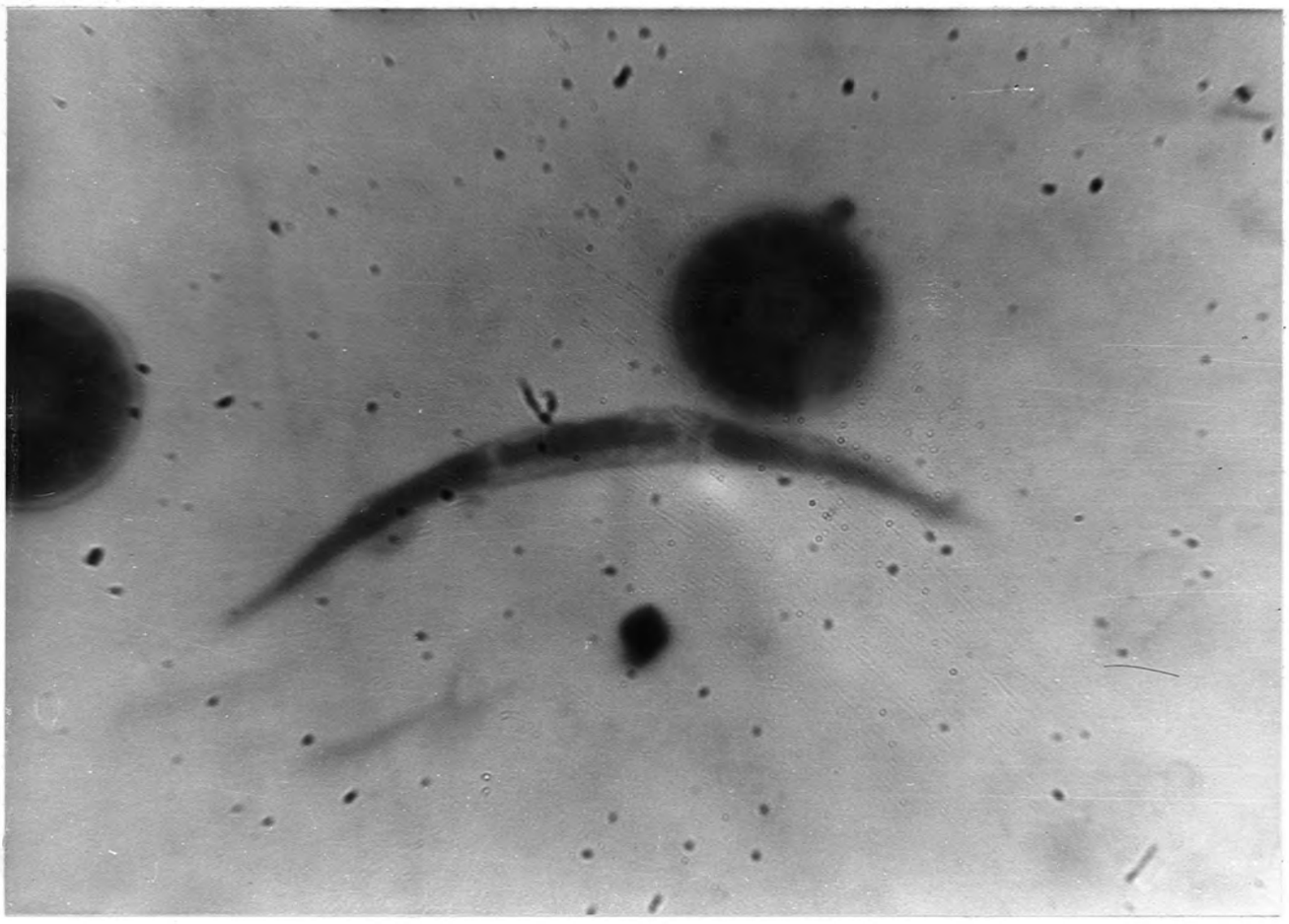

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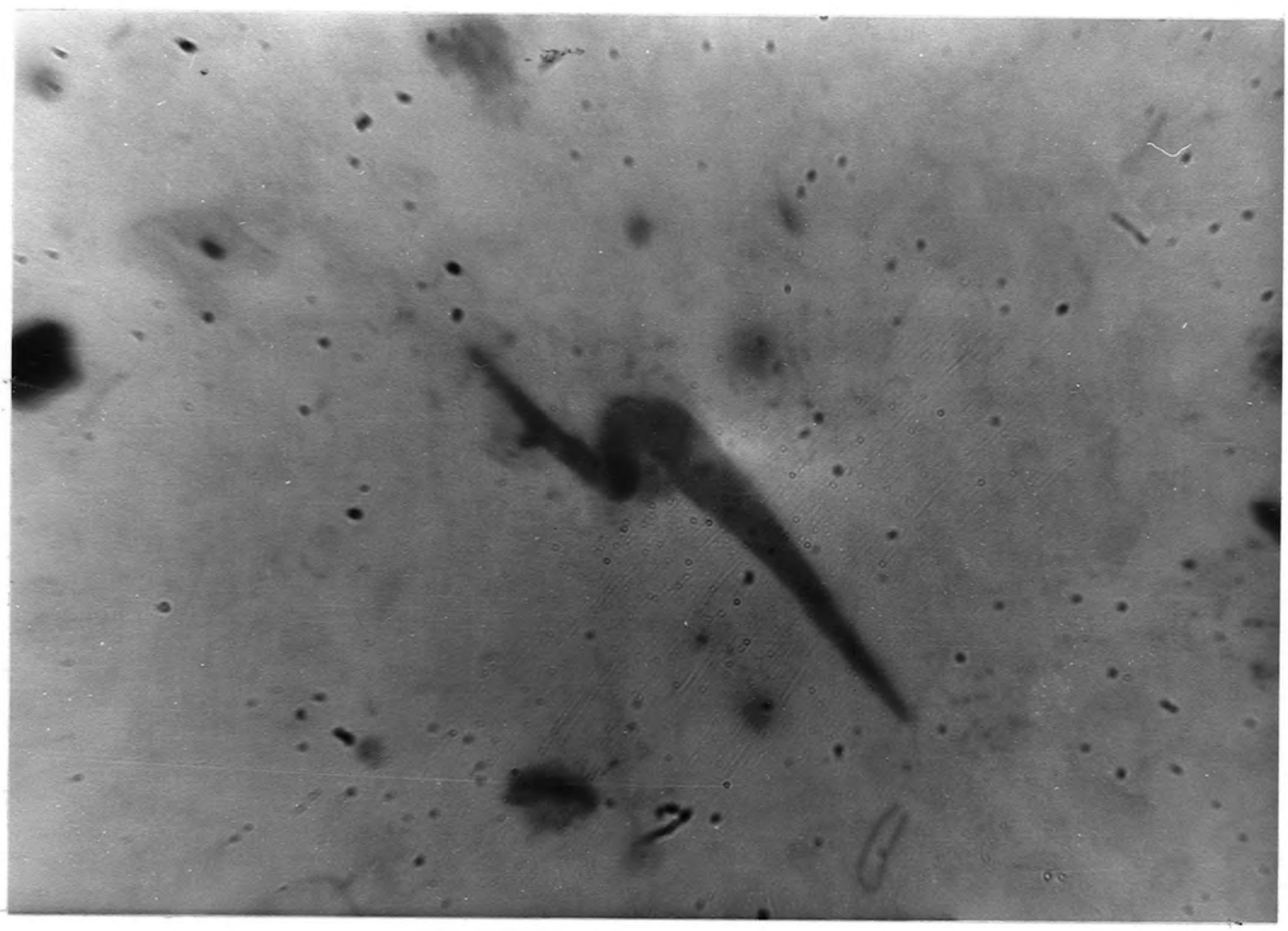

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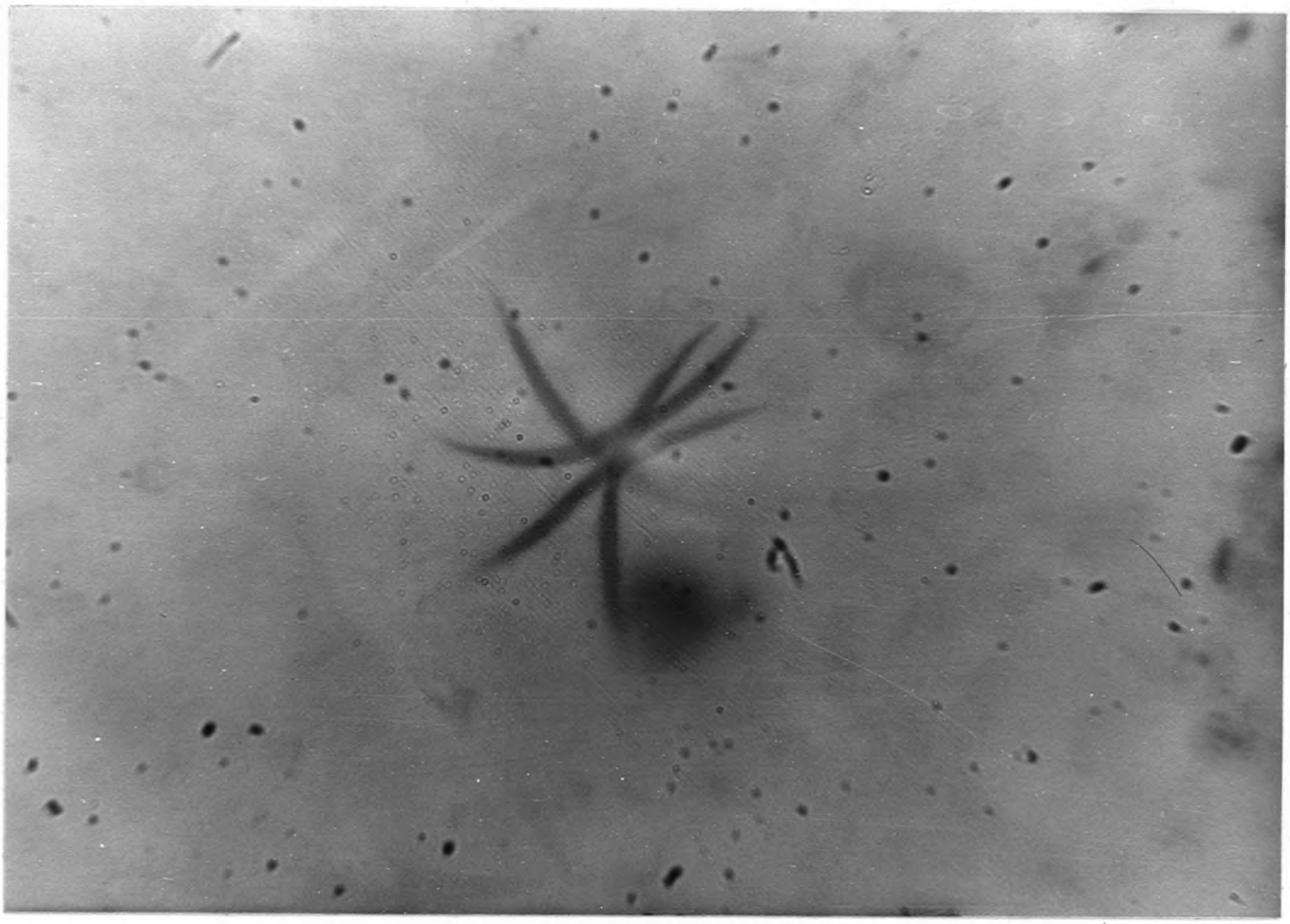

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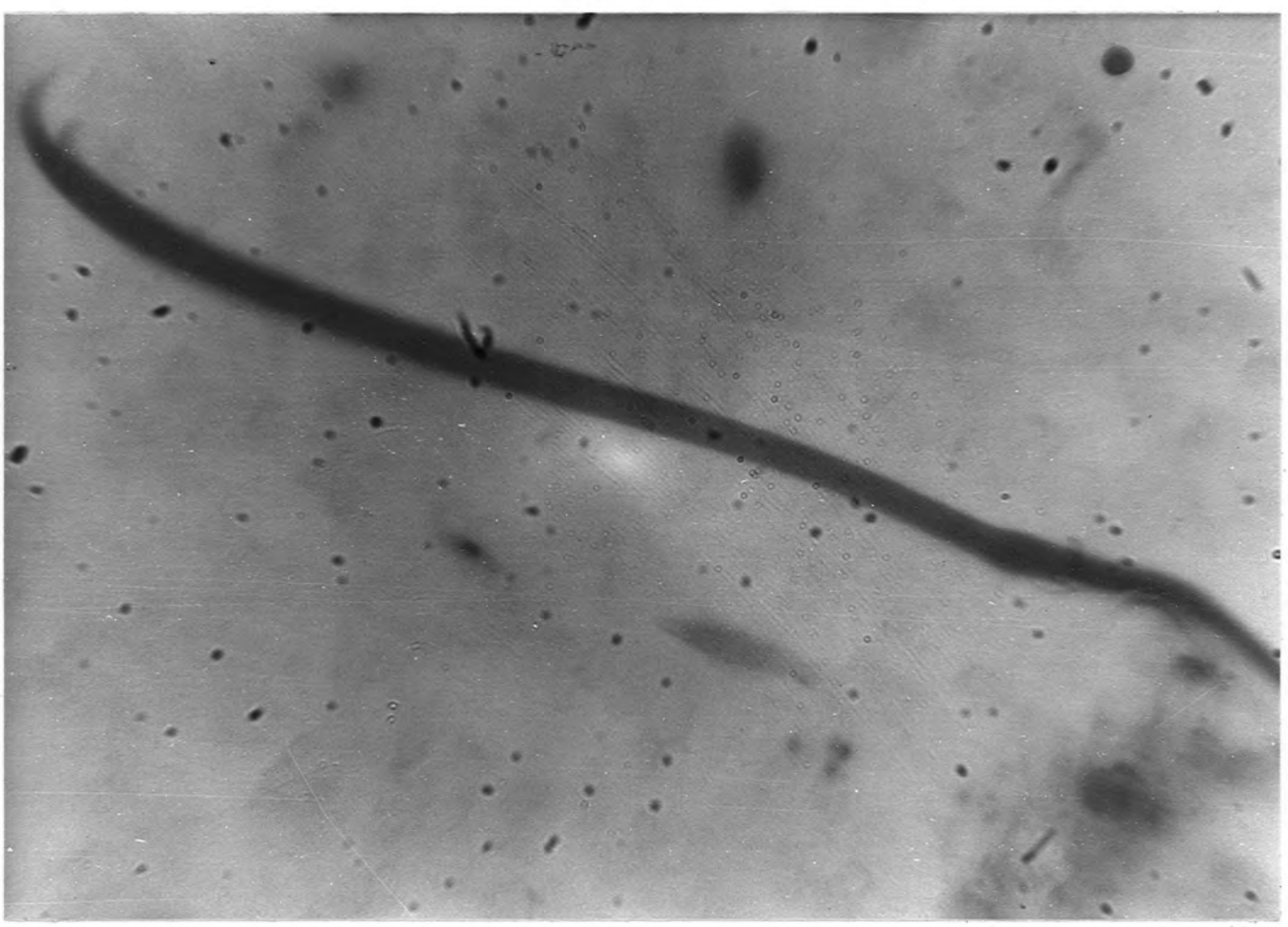

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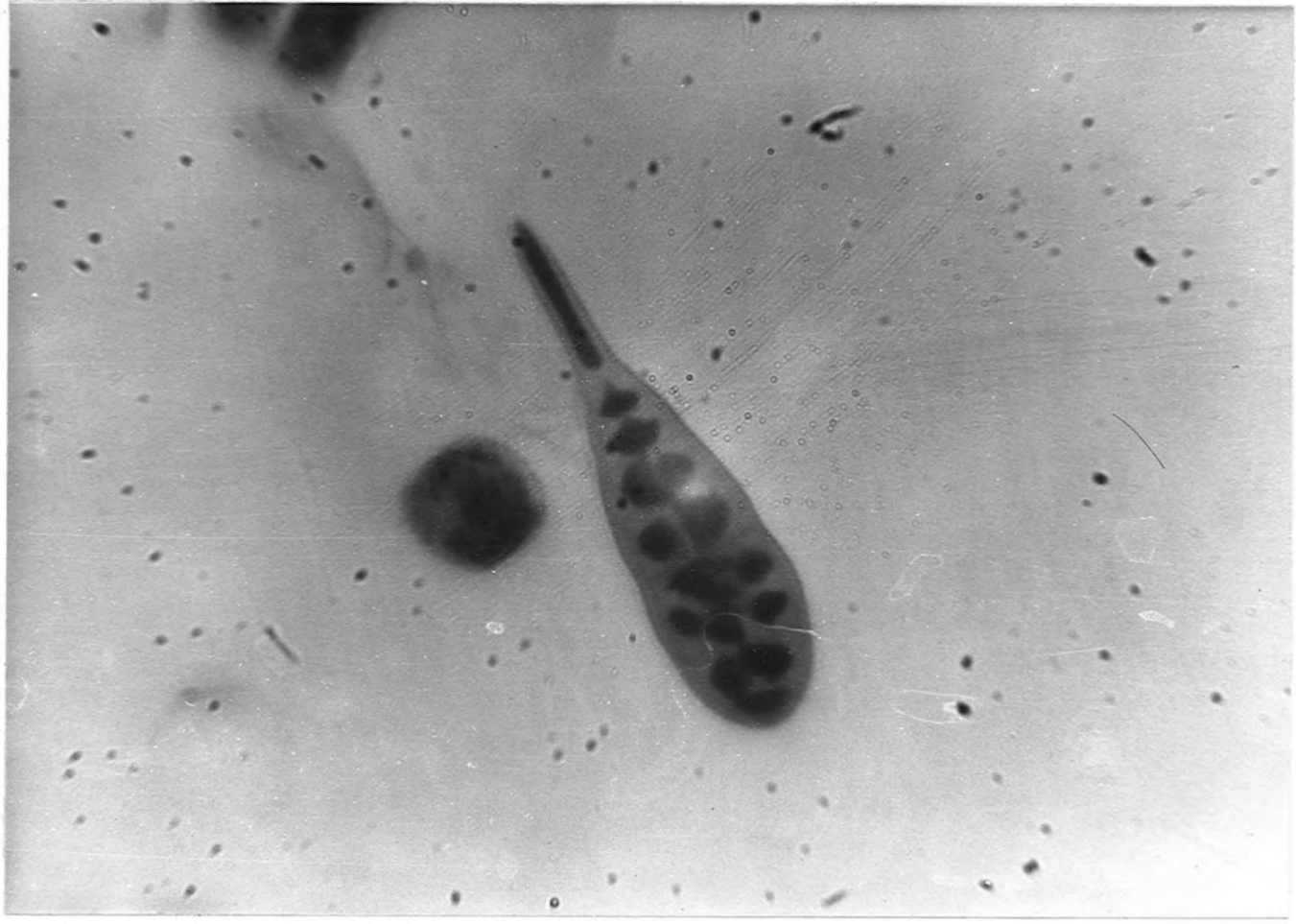

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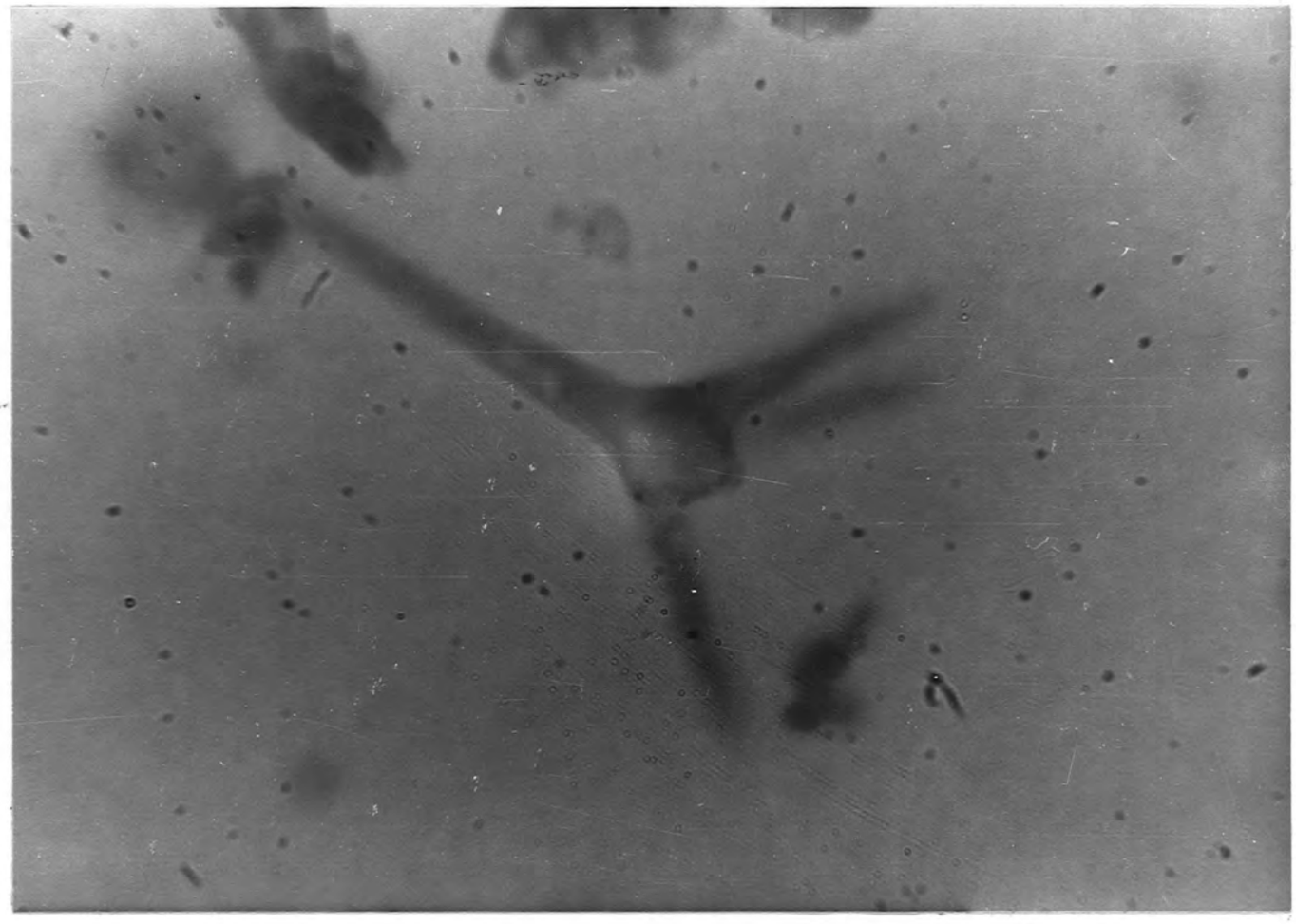

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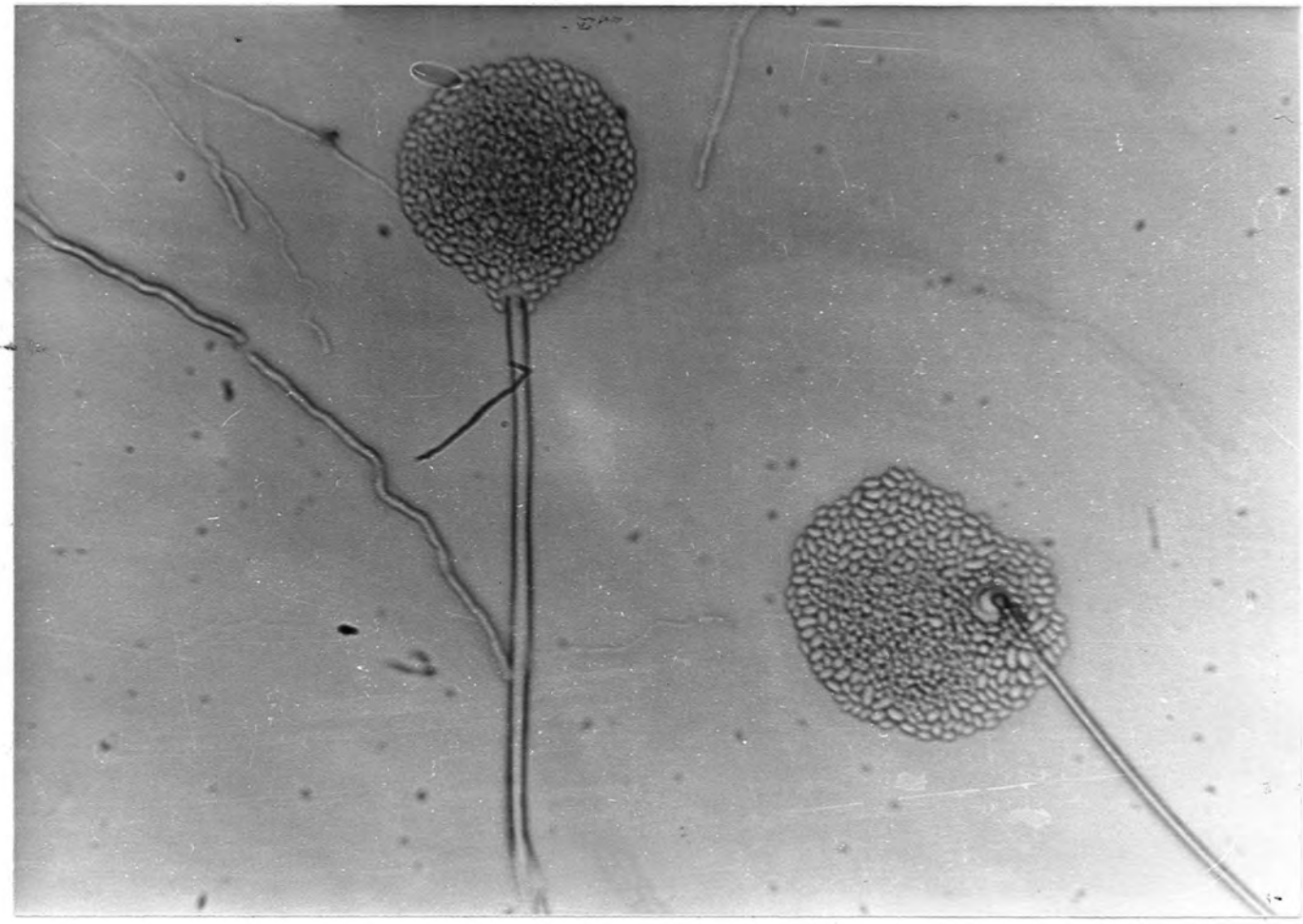

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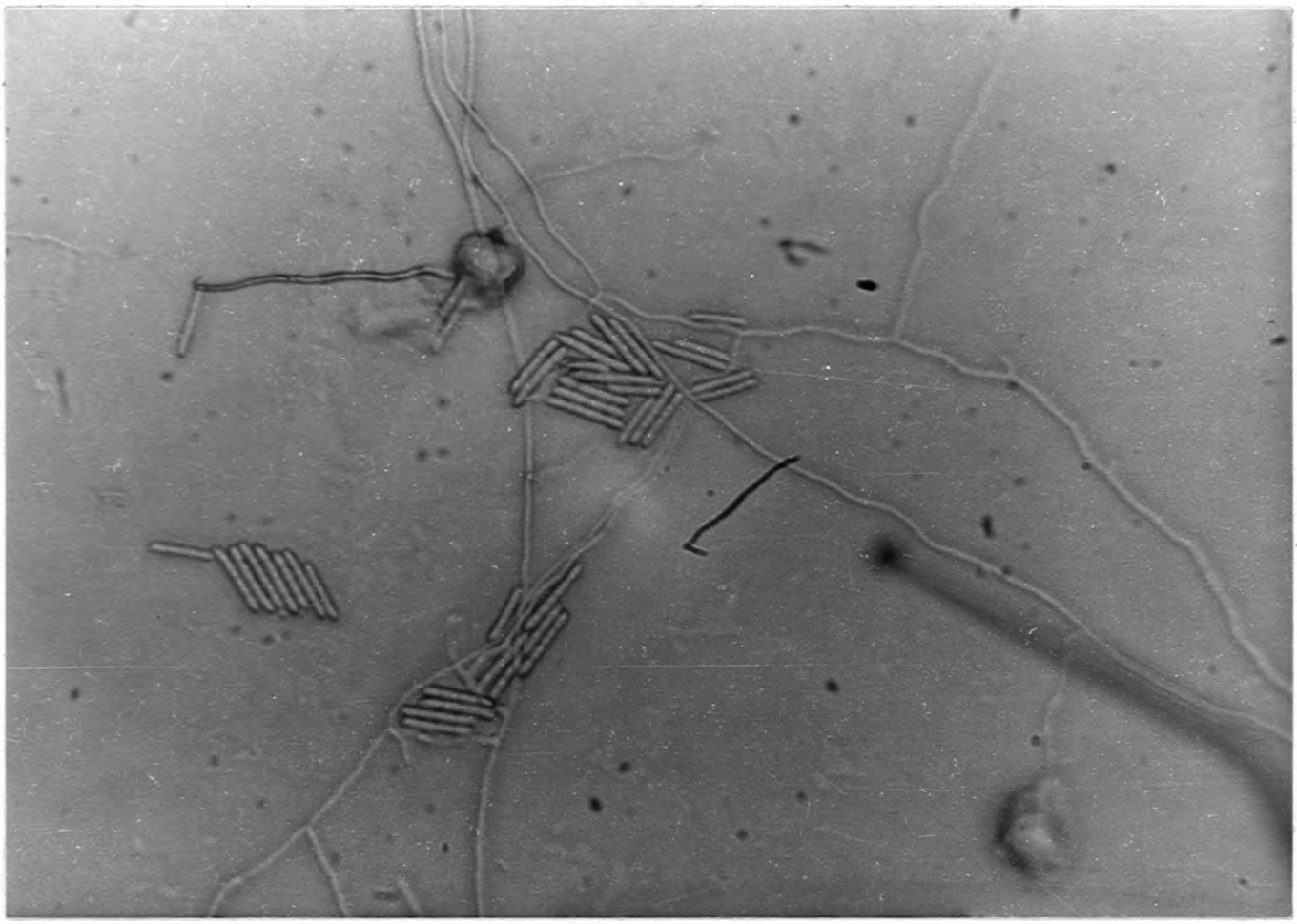

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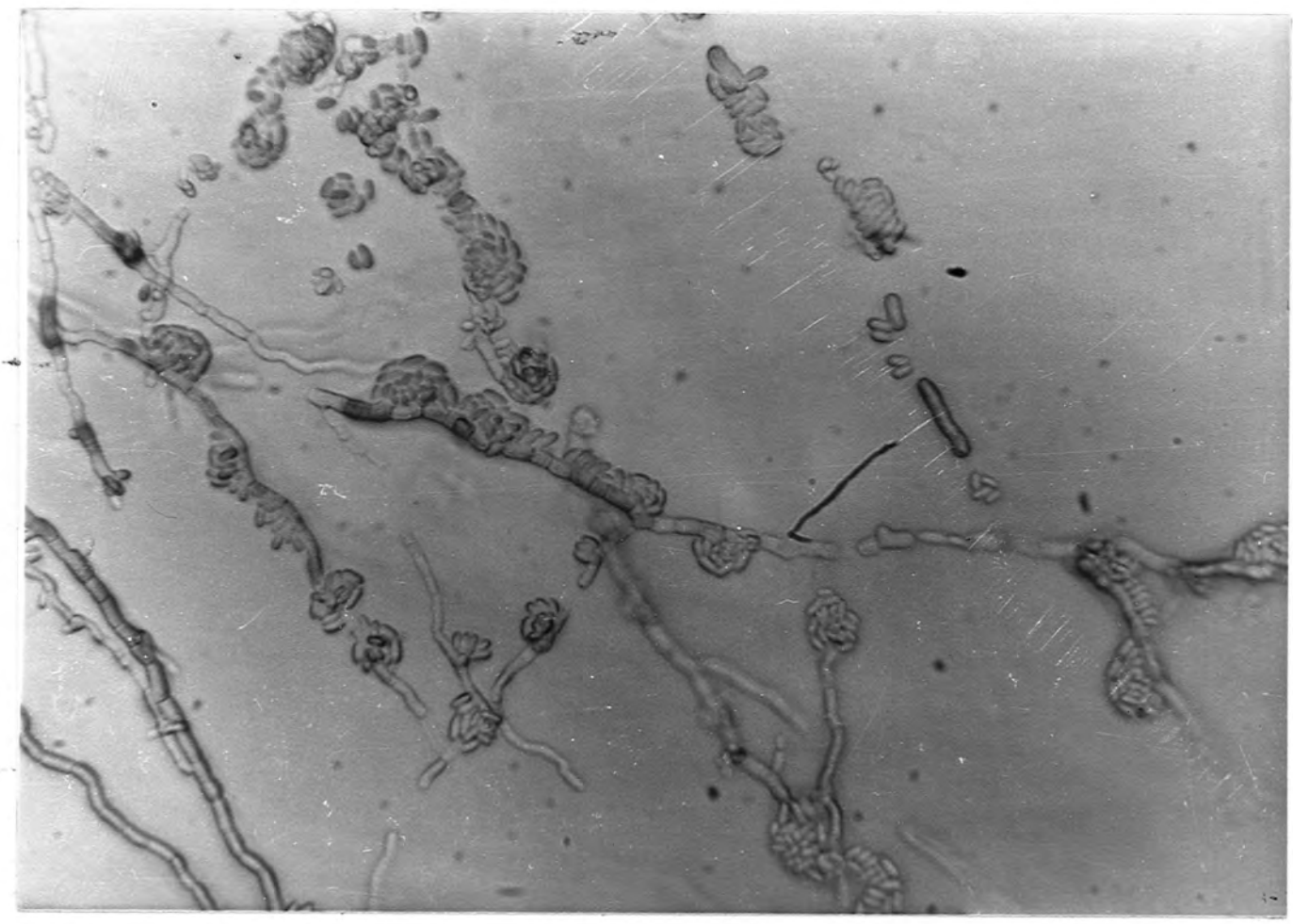


R

Calcarispora hiemalis. (See Fig. 5.1)

Keeping filter volumes constant within each site was not always possible and for ease of scanning it was necessary to alter the volume of water filtered per disc from site to site to accommodate individual problems encountered.

At Site A the filter volume was small because of the huge number of brown and black particles clogging the surface of the filter. Scanning these discs was extremely tedious because of the small number of spores encountered. There were quite a few algal cells though nowhere near the density encountered on the filters from the main river sites. Site A filters were particularly dirty after the heavy rain (24.8.1977). Fungi were always scarce - only 3 of the 5 samples revealed any at all, though the volumes filtered were perhaps a little low at this site.

The filter volume had to be reduced at Site B because of the heavy density of algal cells trapped on the surface of the disc. The algae, dominated by the regular pillbox shaped cells of the unicellular Cyclotella, formed a complete mosaic over the filter. Any spores showed up as slightly denser blue bodies, their branched forms contrasting strongly with the round algal cells. These were few and far between, however, and none were found in the first two samples.

The filter volume used for Site C was a compromise betwwen reducing algal density - which was not quite as high as in B or F - and recording low numbers of scattered spores. Again, one sample revealed no spores at all.

Density of all particles was much reduced at Site D; to make scanning less tedious the filter volumes were increased. Algae were quite abundant again; a variety of diatoms were interspersed within the dominant Cyclotella. Foam sporas were also recorded at this site. Algae did not occur in any numbers in the foam, and amorphous debris and fungal spores made up the bulk of the particles. The record for this site was

complete, in contrast to A, B and C; a greater variety of spores was revealed in the foam than on the filter.

A complete record was also obtained from Site E, the only "large volume" site with a good variety of spores recorded. The filter volumes were kept fairly low because of algae. Again the stacked piles of Cyclotella were dominant.

Site F filter volumes also had to be kept low because of heavy densities of algal cells. Again fungal spores were very few and far between, making scanning extremely tedious. No records were obtained for two of the sample dates.

A complete and very full record was obtained for the woodland stream Site G. Foam and scum were sampled and two extra filter samples were taken, one in July before the main sampling programme got under way and one between the official sampling dates of 18th and 24th August, straight after the heaviest rain. The volume filter varied somewhat from sample to sample. High volumes meant that far too many spores were 'piled' on the disc, overlapping each other and becoming entangled. Algal cells were frequent but fortunately never abundant. This would have led to extremely crowded and diverse discs. This was the only site where algae were not particularly dense and Cyclotella did not dominate the disc.

For the last sample (8.9.1977) the dominant species A-tetracladia was recorded according to its spore form. Thus A-tetracladia f. angulatum (associated mainly with semi-aquatic habitats) and A-tetracladia f. - tetracladia were recorded separately. The split was almost equal: out of a total of 103 spores 50 were tetrahedral and 53 had the Tricladium-like spore shape.

In Table 5.8 a complete species list is presented and records of species from each method are set out clearly for each site, with the total number of species revealed by each technique over the whole sampling period being shown. No indication of abundance is given. Filter

and foam data form the bulk of these records.

In Table 5.9 the most common species recorded at each site for each method is given. Additional information on maximum and minimum abundance is given for both foam and filter. A glance at the spore and species totals for all sites shown in Table 5.5 shows that the maxima and minima for both spore abundance and species diversity (not used in the specialized sense) coincide. That is, the date on which most spores were recorded from Site A, for example, was also the date on which most species were recorded. Conversely the date on which least spores were found was also the date on which least species were recorded. These dates are not the same for each site, however. In fact the maxima for Site E, for example, occur on the same day as the minima for Site G.

5.2.1.3. Leaf Disc Data.

Records of growing and sporulating fungi and of impacted spores observed on the surface of leaf discs preserved before and after lab. incubation are presented in detail in Table 5.6 (at back). Leaf material was collected on two occasions from each of five sites. A separate sub-table is presented for each site showing the species observed on each of the leaf discs subjected to water and ISA incubations, as well as on those preserved immediately for direct observation. Densities are indicated on a scale 0 to 3 (see Table 4.5). Observations from the solid ISA plates after the leaf material had been removed are also included.

In Table 5.7 this detailed information is condensed. A record of all species observed growing and sporulating on the leaf discs ('loose spore' records are not included) is given for each site, treatment and stage of treatment. No indication of abundance is given. This table allows a rapid survey of differences in the floras revealed by collection from different sites and by the use of different observation techniques.

Records from leaf disc observations are also included in the complete

species list given in Table 5.8. Species found sporulating on discs or on plates are indicated for those sites where leaf collections were made. No indication of abundance is given.

In Table 5.9 the most common species from each observation technique is recorded for each site.

Several difficulties were encountered in obtaining fungal data from the leaf discs.

I had realised that shortage of suitable material would lead to leaf discs of rather mixed condition being scanned. For a particular site on a particular date 'mixed' samples were prepared with discs of different condition being distributed as evenly as possible between the various treatments and treatment stages. The proportion of 'ideal' tissue (rather thin or skeletonized) differed from site to site and on the two sampling occasions, however.

Much larger samples (collected and/or scanned) would have ironed out some of these differences and made the records more representative and a more rigorous type of comparison possible.

Ease of scanning varied greatly. This could be due to original leaf condition, site condition (esp. Site A) or incubation conditions.

For instance, the leaf discs from all sites were either thick and blue staining - perhaps with patches of skeletonization; almost completely skeletonized; or of thin yellow tissue. The blue staining spores and sporophores projected into the well illuminated spaces of the skeletonized tissue and were easy to spot and identify. Blue stained sporophores and spores showed up sharply against the reasonably well illuminated thin yellow tissue. However, these blue objects were rather difficult to see against a blue staining background which did not allow much light to pass through.

The distinctive shapes of the aquatic Hyphomycete spores did help somewhat in picking them out against a background of blue leaf cells but it is likely that some were missed. The importance of this may be diminished somewhat in that such material bore a very sparse flora (if at all). It would only be a few scattered sporosphores and spores that were missed. Dense groups ware rare and easy to pick out.

Site A leaf discs were extremely difficult to scan. In spite of careful washing, as layer of fine debris remained on the surface, having been trapped there from the dirty water. A larger proportion of the leaves recovered were thick with very small areas of thinner or skeletonized tissue.

There were also difficulties which arose during incubation. No antibiotics were added to the solid ISA plates. This was not important in those plates incubated for only three days, but occasionally bacteria affected the discs and plates incubated for the longer periods. In some cases parts of the discs and the plate were obviously overgrown by bacteria. In others the bacteria involved was capable of eroding the agar beneath and around the disc. It was obvious that the bacteria had been introduced with the leaf because of the very localized erosion in the immediate vicinity of the disc which sank into the pit thus formed and was extremely difficult to get out undamaged. This happened to material from each of the sites and is indicated on the tables.

Another problem, not appreciated until too late, was the proliferation of algae on a few of the leaf discs incubated for the longer periods. The constant temperature room was permanently illuminated and I had not taken the precaution of covering the incubated plates.

This was a problem at Site A, with leaf discs from the 7 and 10 day incubations having obvious surface algal growth. This may have obscured or competed with the fungi to a certain extent.

Obtaining data from Site A was therefore a particularly daunting task

with thick leaf tissue, embedded dirt and bacteria and algae either obscuring the fungi or restricting their growth.

Some of the Site C discs (especially from the first sample) were affected by bacteria. Unfortunately the direct observation discs scanned from the second sample were all very thick and no spores were observed, although the number to be observed was probably very low.

Site D leaf discs revealed a rather different flora to that of the other sites. Many of the discs cut from the 24.8.1977 sample were not skeletonized but very thin and yellow. It was particularly obvious from these easily scanned discs that T. marchalianum grew profusely on the lamina of the leaf whilst the other species observed on discs from this and other sites grew from the network of veins. It was also very obvious from this sample which of the sporulating T. marchalianum observed were part of 'established' colonies and which had developed from spores which had germinated during incubation. The established colonies were compact little clumps whilst the germinating spores produced scattered, solitary sporophores, not aggregated into groups. This germination was observed on the day 7 and 10 ISA incubations.

Site E material differed from that of other sites; all of it was very thin and skeletonized. In fact some was so delicate that it had to be cut into pieces with flamed scissors; punching discs was not possible. This meant that the disc and pieces were fairly easy to scan but extremely difficult to handle. Bacterial erosion, especially on ISA day 10 incubations made it almost impossible to remove the material from the plane. Algae proved a problem, but only on water incubated discs from the second sample.

A much wider range of fungi were recorded from Site G. Although some bacterial erosion affected day 10 ISA incubations, there was no obscuring of discs by algae or debris. Quite a mixed sample of material was obtained from both collections; this was either fairly thick and blue or

well skeletonized but not too delicate. Some of the details of leaf disc condition and the abundance of fungi recorded are noted below:

1st sample, 24.8.1977

Direct observation:

D1, D2 - aquatic Hyphomycetes recorded, leaf material well skeletonized.

D.3, D4, D5 - aquatic Hyphomycetes not recorded, leaf material blue staining and entire

Water incubation:

Day 3 D1, D2, D3, D4 - good aquatic Hyphomycete records, leaf material well skeletonized

Day 7 D1, D2 - aquatic Hyphomycetes recorded, leaf material well skeletonized.

D3, D4, D5 - no aquatic Hyphomycetes recorded leaf material thick and blue staining

5.2.2 A more detailed look at the field study data: potential and actual comparisons.

5.2.2.1 Introduction

In 4.1 certain aspects of the practical study of fungal ecology were discussed, along with special problems encountered in the study of microfungi in general, and aquatic Hyphomycetes in particular. The practical and theoretical considerations which influenced the construction of the present field and laboratory programmes were set out in section 4.2, whilst various factors which affected collection of both materials and data during the course of the study were commented on in 5.2.1.

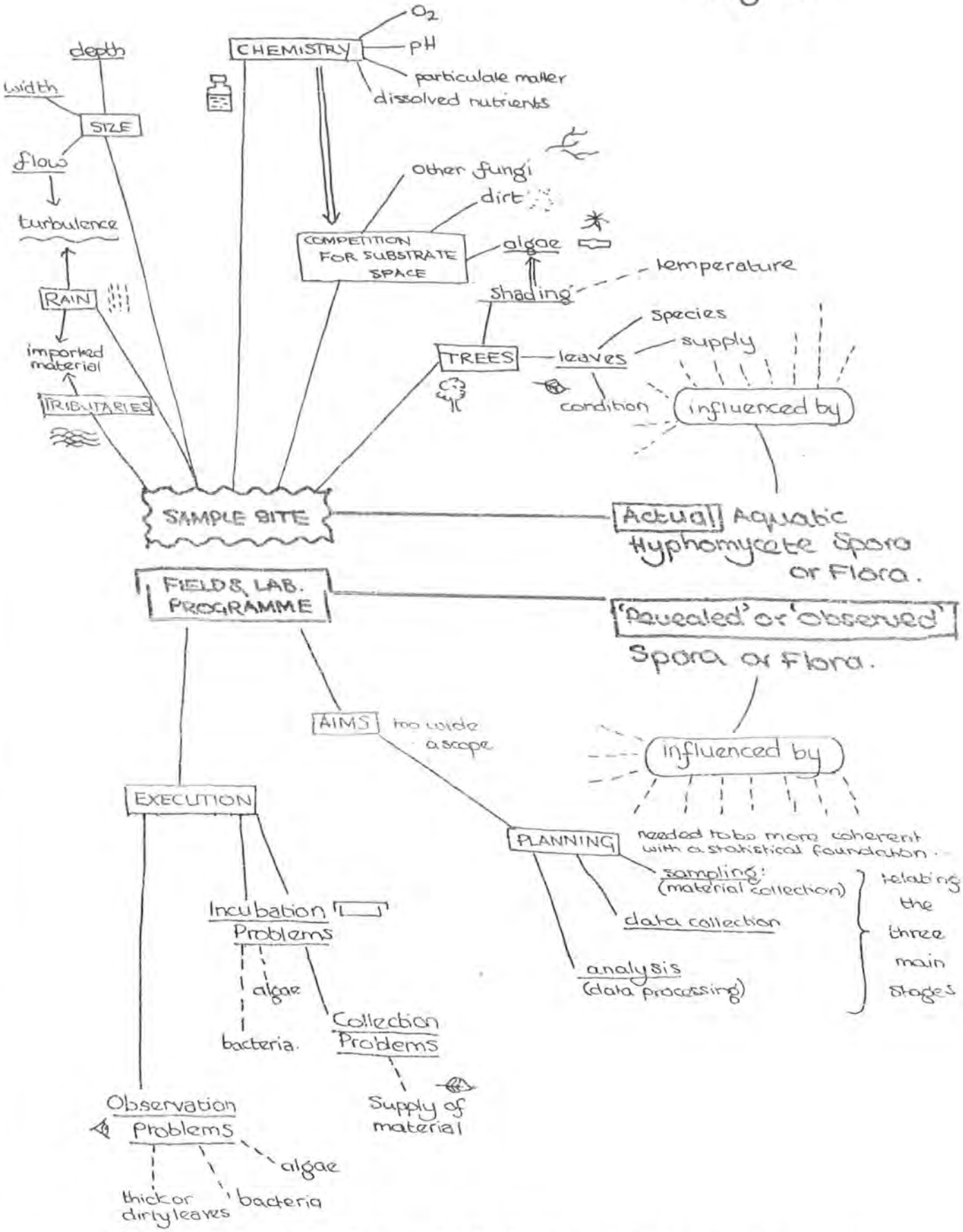
All of these fators and considerations affect the usefulness of the data collected (see Fig.5.2). They place limits on how rigorously comparisons can be made, and what confidence (in both the statistical and everyday sense) can be placed in any of the conclusions drawn.

One of the major factors limiting the usefulness of the data emerged after I had compared the practical work and had had time to consider both the data and the literature more fully.

Since too many comparisons had been envisaged, the data was rather thinly spread out, with a small volume of information on sporas and floras being available for several different sites on several different dates. No statistical framework had been built into the programme. The inclusion of statistical considerations at the planning stage might have produced a more concise programme. As it was, decisions on the number of sampling dates, the number of sites sampled, the number of leaves collected, etc., were based on a combination of practical expediency and a wish to carry out and present an interesting and fairly varied study. Much more thought had been put into the collection of the data than into how it was going to be used to arrive at any significant conclusions.

This lack of a statistical framework, combined with scattered data drawn from highly variable sources over a short period of time, means that the following comparisons are not statistically based. Although certain differences and similarities emerge clearly they cannot be rigorously upheld and the source of such similarities and differences cannot be as easily identified as in a programme where the amount and type of data collected is related to its ultimate analysis, and where various known and unknown sources of variation are eliminated or taken into account.

Fig. 5.2



Some of the factors which influence the composition of both the actual flora and spora of a river or stream and those revealed by field sampling and laboratory observation.

5.2.2.2 Comparisons between sites

Certain comparisons were envisaged when the present field investigation was planned, influencing the way the programme was constructed (4.2.). The most obvious of these were the comparisons that might be made between various lotic sites selected for study. It was thought that differences in the character of the sites might be reflected in the aquatic Hyphomycete floras and sporas they were found to support.

Seven lotic sites were chosen (4.2, 4.3). Two of these were located on the River Wear itself (Sites B and F), two on tributaries close to their confluence with the Wear (Sites A and C), two on tributaries further upstream from the Wear (Sites D and E), and one on the 'self-contained' stream in Little High Wood. Certain differences in width, depth and volume were obvious at the start of sampling, whilst other differences in shading, availability and condition of leaves, and in the quality and chemistry of the water, etc., were revealed during the course of the study through observation, measurement and research (see 4.3 and 5.2.1).

All the sites had to be potentially favourable to the occurrence of aquatic Hyphomycetes and they thus resembled each other in various ways. At or near all the sites tumbled stones or obstructing branches created turbulent flow. At or near each were wooded banks, with various species of tree overhanging the water. Sycamore (Acer pseudoplatanus) was always one of these species and provided the summer-shed leaves collected at Sites A, C, D, E and G. All were situated rather close to each other (see Map 4.2), and with the exception of the woodland stream all sites were on running waters belonging to the same river system.

The data from the various sites on their aquatic Hyphomycete floras and sporas is presented in Tables 5.5 to 5.9. This data can be considered in a variety of ways, as can the information accumulated on the character of each site (see 4.3, 5.2.1 and Table 5.1, 5.2 and 5.3).

Interesting and potentially important information on the overall abundance of aquatic Hyphomycete spores and growing fungi, and on the dominant members of the flora and spora, can be culled from the various aquatic Hyphomycete records.

The composition and abundance recorded at each site were not those of the actual flora and spora, but provided more or less accurate reflection of the true situation. The accuracy of this picture depends on a combination of many factors, such as appropriate choice and range of observation methods and availability of suitable material, etc. (see Fig. 5.2 and 5.2.1). The composition of the actual aquatic Hyphomycete flora and spora depends on a complex of site characteristics, some of which are set out in Fig.5.2 and some of which were observed and recorded in this study.

In searching for possible explanations for differences observed in the aquatic Hyphomycete composition of the various sites, both the site characteristics and 'experimental influences' must be taken into account or at least kept in mind. For example, the dirt covered leaf material retrieved from Site A was extremely difficult to observe. This may have prevented some of the fungi present on the surface from being detected In addition, the competition for substrate space experienced by aquatic Hyphomycetes trying to colonize such dirt collecting surfaces may have discouraged germination and growth.

In getting some idea of the general abundance of aquatic Hyphomycetes, both as spores and as growing fungi, the most useful data are those presented in Tables 5.5 and 5.6.

It is immediately obvious from the filter records (5.5) that spore numbers (per unit volume of water) were far greater for the woodland streamsite, Site G, than for any of the other sites. In spite of large variations - from 24 to 824 spores per 100ml filtered- the densities from this site were consistently higher than those recorded from any of the other sites.

Sites B and F and Sites A and C, never produced densities higher than 10 spores per 1000ml.(40 per 400ml.) less than half of the minimum recorded for Site G. Also, none of these sites had complete records; on one or two occasions no spores were recorded at all.

Spore numbers for Sites D and E were a little higher, and their records were complete. Here the highest densities were 106 per 400ml. (Site D), and 116 per 400ml., about the same as the minimum densities recorded for Site G.

Looking at the leaf disc data it can be seen that the number of leaf discs colonized by aquatic Hyphomycetes, the number of species found, and the densities at which they were found, all combine to give an idea of overall abundance.

Here growing fungi are being considered (although loose spores observed on the leaf surface are recorded, as '0'). The best picture of the active flora is likely to be given by the records from direct observation and the 3 day incubations.

Differences between sites, in terms of abundance alone, are much less pronounced than in the spore data. Site A, however does appear to have a particularly meagre record,whilst Site G boasts better records for the direct observation and 3 day incubated material than do the other sites.

In considering the species composition of the floras and sporas of the various sites, information on the number and identity of the species found is given in Table 5.8, whilst information on the relative importance of the various species can be gleaned from the detailed records presented in Table 5.5 and 5.6.

Only the sporas of Sites B and F are available for assessment and comparison. Seven species were retrieved from B, and six from F, five

of these being in common. Only Tetracladium marchalianum was recorded on each of the dates that species were found.

Although seven species of spore were also retrieved from Site A, numbers were very low and only one, "unknown A" (Fig. 5.3) was recorded on more than one occasion. Only two species were recorded growing on leaf material, T.marchalianum and A.longissima. It is interesting to note that two of the species observed as loose spores on the surface of Site A leaf material were not picked up on the filter discs. Presumably the density of these species was too low to show up in the volumes filtered.

Site C's spora was not only sparse in numbers but also in species, only four being found. However growth and sporulation of aquatic Hyphomycetes was much more vigorous at this site than at Site A,and three species were recorded, T.marchalianum, Anguillospora sp. and C.aquatica.

The data from Site D are a little more interesting. Here the filter records of the spora are augmented by observations from foam. Seven species were detected on the filter discs during the course of the study, whilst nine species were concentrated in the foam. Of these only three species were in common, the other six were not picked up by filter sampling, presumably because of their low concentration. T.marchalianum was again rather common, being present in all but one of the filter and foam samples, and being the most abundant in the majority of these.

T.marchalianum was also found growing on the leaf discs. Table 5.6d shows that four species were observed on Site D leaf material, T.marchalianum and Tricladium angulatum being rather more common than the others.

Table 5.8 reveals quite a large species list for the Site E spora, much larger than any of the other tributary sites for. However, T.marchalianum, the only species recorded on each of the 5 sampling occasions, was also the only one whose spores were found in any great number. Site E leaf disc incubations suffered more than the material from other sites from algae and bacteria. Four species of aquatic Hyphomycete were recorded growing on the leaf material; T.marchalianum, again, had the largest number of records.

Not only was the abundance of spores revealed by both filter and foam sampling much greater for Site G, but the number of species making up the spora was much higher at this site than at any of the others. In all, 21 species were found on filter discs whilst 12 were recorded from ('new') foam; 11 of these were in common.

Another difference in the spora of Site G and apparent from examination of Table 5.5g, is that several species make up the bulk of the numbers found. Heliscus lugdunensis, Tricladium splendens, Clavatospora stellata and Articulospora tetracladia were particularly important. Not only did these appear in large numbers, but apart from one occasion on which no H.lugdunensis was found, all four of these aquatic Hyphomycetes appeared in every single foam and filter sample taken.

The abundance of sporulating fungi observed on Site G leaf discswas not very different from that at other sites. However, it is interesting to observe the rather more prolific growth of both aquatic Hyphomycetes and terrestrial fungi on the 1SA incubated leaf discs for 9:9:77. This was presumably partly due to less interference from bacteria and algae.

The range of species found at Site G was much wider, nine aquatic Hyphomycete species and six species of terrestrial imperfect fungi being recorded. Tricladium splendens was the species which appears to have been the most successful on unincubated and water incubated material, whilst Tricladium terrestre was the 'aquatic' Hyphomycete to show the most growth on the 1SA incubated material.

Certain common features of the floras and sporas of the various sites can be picked out from the data summarized in Tables 5.8 and 5.9. T.marchalianum, for example, occurred in every single flora and spora and its importance is further emphasized by its being recorded 16 out of a possible 24 times in Table 5.9, noting its absence from the Site G entries. No other species was recorded more that twice. Anguillospora also had a fairly consistent record, being found in all but one of the sporas (Site D) and all but one of the floras (Site G). Heliscus lugdunensis

appeared in the spora from all sites but was never recorded sporulating on leaf material.

What features of the sites can be used to help explain the apparent differences and similarities set out above? Not forgetting that some of these may have been exaggerated or diminished by sampling and observation techniques and difficulties,and that some important parameter influencing the composition and abundance of aquatic Hyphomycete floras and sporas may not have been measured or observed. Table 2.6 (at the back of the thesis, and abbreviated to 2.6) will be useful in this discussion.

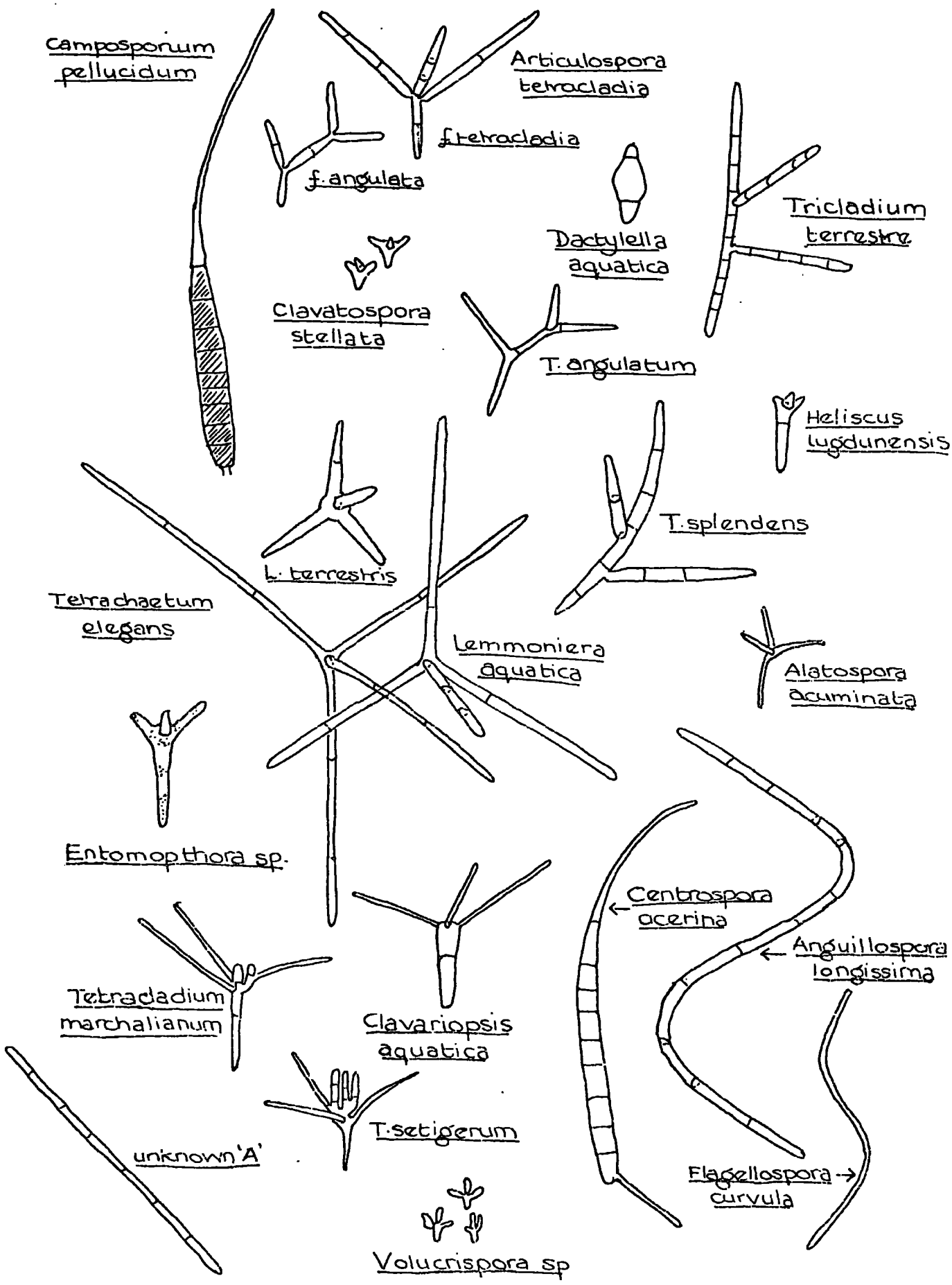
The waters sampled at Site G supported the most varied flora and contained the most abundant amd varied spora. There are several features of this stream which might help explain this richness.

A low volume, self-contained woodland stream with no tributaries, it was completely overhung with trees and the water was relatively clear. Algae and dirt, therefore,did not compete overmuch with fungi for substrate space. Shading also kept the temperature rather lower at this site, which may have favoured aquatic Hyphomycetes, as may the lower pH of the water. The supply of leaves relative to the volume of water was much greater than at any of the other sites, the whole bed and bank being covered with various species of leaf. Runoff was from steep grass and leaf litter covered banks. All of these features may have contributed to the success of aquatic Hyphomycetes at this site. Certainly a combination of factors has produced a rather different flora and spora from that supported by the other, less rich sites.

The spora is very large. Not only that, but some of the largest contributors are not members of the revealed flora of the sycamore leaves; H.lugdunensis and C.stellata, for instance . There are two possible explanations, which are not mutually exclusive. Either the spores have been washed in from the wooded banks which border the stream or they have been released from the flora of other species of leaf submerged in the stream.

Spora of Woodland Stream site G

Fig 5.3



There is no way that the relative contribution to the spora of the floras of stream and bank can be assessed from the present data. If Nilsson's (N64) contention - that the spores of A.tetracladia f.tetracladia are produced submerged whilst the spores of A.tetracladia f. angulatum are produced aerially - is true, then the 50:50 split between the two observed on 8:9:77 suggests a 50:50 contribution,at least on that date.

Heliscus lugdunensis is another 'versatile' species and some of its spores may have been produced on land. Certainly the projections on the clove-shaped spores were not always equally well defined.

All four of the species found to be common in the Site G spora are well known and common aquatic Hyphomycetes, A. tetracladia and H.lugdunensis are particularly successful,being versatile species found in a variety of aquatic habitates as well as land. Nilsson and Triska (2.6) mark A.tetracladia down as a common and constant species, as do Koske and Duncan in their list of woodland species. H.lugdunensis was one of the most common and 'ever present' species in the presumably rather similar self-contained woodland stream in the Lake District studied by Archer and Willoughby (2.6), whilst Bärlocher and Kendrick found it to be one of the most important species in their study.

C.stellata is a temperate species which was reported from summer-shed leaves in Triska's study (2.6). T.splendens was one of the common species in Willoughby and Archer's woodland stream, whilst Nilsson reports it as one of the most common species in Sweden, and Koske and Duncan list it as a common and constant species in woodlands.

It is possible that the large number of A.tetracladia spores found at site G,in contrast to its virtual absence from the other sites, may be due to the lower pH at Site G. Iqbal (1972) reports that the optimum pH for sporulation in this species is 6.5-7.5. The pH at Site G was always found to be 7.5, whilst the values for the other sites were always higher, ranging from 7.8 (Site B) to 8.9 (Site E).

The contrast between the waters sampled at Site G and those sampled at the rest of the sites is reasonably obvious. However there are certain smaller differences between the remaining (river system) sites.

The tributaries sampled at Sites D and E appear to have supported a rather richer flora and spora than the tributaries sampled at Sites A and C, in spite of the fact that E and C are on the same river (see Map 4.2).

Both these sites were well overhung with trees, and the turbulence was much greater than at any of the other sites. The 'revealed' flora of Site E might well have been richer if the leaf material had not been so delicate,and incubation so affected by contamination. The actual flora at this site was probably reduced somewhat by the very obvious and rapid consumption of leaves by invertebrates. This was the site at which most animals had to be removed from the leaf material, which was, as mentioned above, beautifully skeletonized.

The two less rich sites, A (on Croxdale Beck) and C (on the River Browney) were situated on tributaries much nearer to their confluence with the Wear (Map 4.2.). The volume at these sites did not appear to be that much greater than that at the upstream sites, and this does not appear to be the cause of the difference. Certainly at Site A, the fine particulate matter blanketing the leaves is likely to have severely reduced the possibility of aquatic Hyphomycetes germinating and growing on the leaf surface (NG4). It is much more difficult to put forward an explanation for the reduced spora at Site C. The only obvious difference between this and other sites was a slight reduction in the availability of oxygen (and that was the mean over the year, not measured at the time of sampling).. Also the fact that the banks at the site itself were clothed with dense stands of Impa tiens (see Fig.4), the overhagging trees and grassed banks being just upstream of the site itself. Not quite as many leaves were ever found at this site as at the others.

The two sites, B and F, located on the River Wear itself show very low numbers of both spores and species. Here the cause is likely to be the relatively hugh volume of water receiving and 'diluting' spores washed in from banks and swept in from tributaries. No leaves were collected and observed so no assessment of the flora at these sites is available.

The three most common species from the River Wear system sites were T.marchalianum, Anguillospora sp.and H.lugdunensis. T.marchalianum was important in the combined flora and spora of these sites, Anguillospora was important in the flora, whist H.lugdunensis was a consistent member of the spora only. The importance of this latter species has been discussed. The obvious success of T.marchalianum in this particular investigation may well have been slightly over emphasized due to its presence on the leaf discs and medium of the 1SA incubations. It may be that this species sporulates more readily than certain others under these conditons.

In 2.6 it is recorded as a common species in nine out of the ten temperate aquatic lists, where it is listed as a constant species, being particularly common in summer. This fits in with its presence in these late summer records. Probably all the Anguillospora recorded were A.longissima but it is difficult to be sure from treated material and without detailed measurement and comparison, certainly A.longissima is also a well known and common species,being recorded in 5 of the 10 lists in Table 6.

Iqbal (1972) suggests that these two flora species,A.longissima and T.marchalianum,are capable of colonizing new leaves using their simple carbohydrates, moving on to an exploitation of the more resistant components as decomposition proceeds. This would help explain their presence on the fairly recently shed summerleaves retrieved in this study.

5.2.2.3 Comparisons between dates.

Since this was a rather short term study (10:8:77 — 8:9:77) it was unlikely that any seasonal trends would be apparent in the fungal records, despite the approach of autumn. In fact, practical work was completed before the major autumn leaf fall had really started. The leaves trapped and collected at each site could all be described as summer-shed and the number of leaves available for collection did not noticeably increase as the study progressed.

However, examination of the data presented in Table 5.5. shows that quite large fluctuations occurred in both the number of species and the number of spores recorded for a particular site on the different sampling dates. For instance, a look at the filter records for site G (Table 5.5g) shows that the number of species recorded ranged from 7 to 18, whilst the number of spores retrieved (per 100 mls.) ranged from 24 to 828.

These maxima and minima in both numbers and diversity are set out in Table 5.9. On the whole each site appears to have reached these independently. Of course more detailed records from larger volumes of water might have refined the picture. Some of the variation may well have been exaggerated by the relatively small samples taken.

One factor which might have been expected to influence variation in both species and numbers at all sites, was the amount of rainfall. All sites were close to each other and to the University Observatory where rainfall was recorded. They all therefore experienced the same amounts, or lack, of rain.

Rain can augment the aquatic Hyphomycete spora of a river or stream in two main ways (already discussed in 2.2 and 2.3). Turbulence can be caused or increased by rain falling directly in the water or by increase in flow, and may stimulate spore production. Rain can also increase both the number of spores and the number of species entering a stream in the run-off from adjacent terrestrial habitats. This

contribution would obviously be greater from the leaf litter layer of a wood than from grass or a cultivated field.

The actual effect of rain on the spora of a river or stream would depend very much on the balance between the increase in volume of water due to the rain, and the increased spore load due to run-off and turbulence.

In a river such as that sampled at sites B and F there may be a large increase in the absolute number of spores washed in from the various tributaries, but the extra volume of water may be such that the effect is one of dilution; the number of spores per unit volume being, in fact, decreased.

However, in a small woodland stream (such as that sampled at site G) where the increase in volume is from runoff alone, the increase in the number of spores and species due to turbulence and runoff may be balanced against a relatively small increase in volume, and the number per unit volume may increase.

The filter records from each site are not equally reliable or enlightening. The most interesting figures to compare are those for 18:8:77 and 24:8:77. Nearly 20 mm of rain fell between these two dates, after quite a long period without prolonged rain.

Site A appears to show a dilution effect, whilst sites B and C show an apparent increase in numbers per unit volume. Site D shows a very marked decrease in numbers, its maxima occurring before the rainy period. Site E shows a marked increase, its maxima occurring after the rainy period. Site F shows an apparent increase, as does Site G immediately after the heaviest rain (in the extra sample taken on 21:8:77); by the regular sampling date (24:8:77) these numbers have declined quite sharply.

Although the numbers of both species and spores increased after rain at Site G, the maxima were recorded on 16:8:77, after the dry spell. The 'rain' increase could be explained by an increase due to both runoff and turbulence. Perhaps the 'dry' increase was due to a concentration of spores such as the volume of water decreased. Such a small stream is extremely sensitive to changes in weather. The volume and rate of flow fluctuated rapidly and widely with the amount of rainfall.

The above is a plausible explanation for variation between and within sites but cannot be proved. A long term study, which compared rainfall with spore load might reveal a pattern for a particular river or stream site. However, other influences - particularly seasonal ones - might swamp the effects due to rain.

It is interesting to note that although fluctuations in the numbers of species and spores did not coincide at the various sites, there was a synchronized effect on the density of algal cells observed on the filter discs (Sites A to F).On 1:9:77 after a fortnight of rain, there was a marked reduction in the density of algae. This was probably due to dilution, though grey skies may have contributed. Site A's filters also showed an increased density of dirt particles, presumably due to the increase in turbulence observed after this rainy period.

5.2.2.4 Comparisons between methods.

No single method of sampling and observation can provide a comprehensive and unambiguous survey of the aquatic Hyphomycete species to be found at a particular river or stream site. It was for this reason that a combination of methods was chosen when the sampling programme was designed. The combination was intended to be complimentary with some of the information being confirmed by more than one method and some being exclusive to the particular method used.

The filter and foam sampling methods were intended to reveal the spora of the site. The combination of direct observation and short-term incubation of leaf material was expected to reveal those fungi actively growing on the leaf at the time of collection whilst, the longer incubations were expected to give additional information on inactive fungi present in and on the leaf.

Differences between the species lists revealed by each of these methods was therefore to be expected, and it is interesting to observe such differences in the data from the present study. Although the volume of data from most sites is not large, certain differences do stand out. The summarized information presented in Tables 5.7, 5.8 and 5.9 is particularly useful in picking these differences out.

For instance, looking at the complete species record in Table 5.8, it is obvious that a far larger species list is provided by the 'spora' methods of foam and filter sampling.

This is hardly surprising. Sampling the spora is much more likely to reveal a larger number of species than sampling the leaf flora. The spora is an aggregate of material imported from the surrounding area as well as from aquatic Hyphomycetes growing in the immediate vicinity. It includes species washed in from the adjacent terrestrial habitat and

from any tributaries. This difference is particularly marked in the records from Site G. Here 21 different species were picked up as spores by the filter method, whilst only 9 were found growing on the leaves.

A strict comparison between the filter and foam data is not possible. Differences in the proportion of filiform and tetraradiate spores are to be expected with the foam selecting in favour of the branched forms, whilst the filter indiscriminately picks up all the spores suspended in the volume of water collected. To make a proper comparison between these two, however, records of the same order of magnitude should have been made. If the filter had trapped 200 spores then the appropriate volume of foam should have been scanned until 200 spores had been identified. This would also have made comparisons between dates and sites much more revealing.

It is interesting to note that whereas the foam spora for Site G was much less rich than the filter spora, the reverse was true for Site D. Fewer spores were suspended in the water of Site D; the foam concentrated those present, giving a more detailed record. At Site G the spore load was much heavier. Foam accumulates more and more spores the longer it persists. However the foam collected from Site G was always a week old, or less, quite 'new'. The selection of spores trapped by this relatively 'new' foam was much less varied and less representative than the spore load revealed by the filter method.

Although the filter method may miss the less frequent species it seems to have much more to recommend it than the collection of foam, particularly when the interest is in the ecology of the fungi sampled, rather than in the compilation of a species list only. Foam cannot always be found whilst filtration can always be done. Filtration also provides a quantitative, unbiased survey of the spore load allowing better comparisons between sites and dates (see 2.3.6.2).

Examination of the summarized records in Table 5.7, and the more detailed data set out in Table 5.6 reveals certain differences in the floras (and 'sporas') recorded for each of these different procedures. Again, the larger volume of diverse data available for the richer Site G provides the most interesting material for comparison.

Although it is easy to see that differences do occur it is not as easy to attribute them to particular causes. It was pointed out in 4.3 and 5.2.1 that the material retrieved from each site was far from uniform. As far as possible the leaf discs cut were sorted out so that no one treatment would receive all skeletonized leaf discs, for instance, whilst another received all thick, green discs. It is not impossible, however, that in spite of this sorting, the leaf material varied enough to produce quite large differences, sometimes obscuring or compounding variation from other sources.

Table 5.7 shows that the number of species recorded for directly examined leaf material from Sites A, C, E and G was smaller than that for leaf discs incubated in water for three days. For Site D, a greater density of fungi was recorded for the incubated material. This short term incubation was indeed intended to augment the observations made from unprocessed leaf material, by encouraging active fungi to sporulate and be identified. The increases may be due to this expectation being fulfilled.

Although the records for the short and long term water incubations differ they do not do so in a consistent manner. Observations made by Nilsson (N64) and several others suggest a decline in sporulation after a few days incubation in small volumes of water. Some evidence for a decline can be seen in the data for Sites C, E and G but it is impossible to confirm the cause.

There is evidence that some species of aquatic Hyphomycete will not sporulate unless submerged in water, though other work suggests that this may be due to the medium on which the fungi are grown(2.2.2.4). It is interesting to note that although the number of aquatic Hyphomycete species recorded from leaf discs incubated on the solid 1SA medium was lower than from water incubated material, several species were observed growing and sporulating on the medium itself, after the leaf disc had been removed.

A particularly interesting feature of the flora recorded from Site G was the appearance of Tricladium terrestre on both leaf discs and the solid plate. This species is associated mainly with a terrestrial existence, and was not recorded from water incubated material.(2.3.6.1).

More interesting still was the strange appearance of a species of Flabellospora on a solid plate from Site C. This attractive fungus was observed growing and sporulating at a very high density on the 1SA plate itself, but was not observed on any of the discs.

The most obvious differences between the species of fungi recorded from the two types of incubation, was the presence of growing and sporulating terrestrial imperfect fungi both on 1SA incubated leaf material, and more especially on the solid medium itself. There were no records of such terrestrial fungi sporulating on any of the leaf material incubated in water.

The obvious inference is that these terrestrial species were present in an inactive form either as hyphae, or more likely as spores, on the submerged leaf material. All the species recorded are very common phylloplane and litter species and could have either been introduced with the leaf as it fell into the water or washed in as spores and subsequently impacted on the leaf. Incubation on the solid medium then encouraged their rapid germination, growth and sporulation. (3.3.2).

All the methods used to build up the picture of a particular site's flora and spora concur over certain features. This is particularly

evident in Table 5.9 where the most common species recorded by each method for each site is set out. Almost every record for the Sites A to F is of the successful aquatic Hyphomycete Tetracladium marchalianum, whilst the importance of Tricladium splendens in the flora of Site G is confirmed by 2 of the 3 methods employed.

5.3 Conclusions from the Field Study

5.3.1 Biological conclusions

This is a brief summary of the more detailed discussion of the data presented in section 5.2.2., where comparisons were made between the aquatic Hyphomycete floras and sporas revealed at each site, on each sampling date, and by each sampling method. Biological and practical explanations were sought for the various differences and similarities observed, and any conclusions drawn are subject to the cautions expressed in 5.2.1.

Seven lotic sites were studied. Differences were expected between the three main 'categories' of flowing water: main river , tributary and woodland stream. Because each had certain features associated with the presence of aquatic Hyphomycetes, some similarities were also expected to emerge.

The expectations were, on the whole, borne out by the data collected. The self contained woodland stream (Site G) differed rather markedly from the other sites in the relative richness and abundance of the aquatic Hyphomycetes found in its waters. Its spora was dominated by four species, A.tetracladia, H.lugdunensis, T.splendens and C.stellata; whilst T.splendens and A.longissima were important in its flora. The relatively large supply of leaf substrate was probably one main factor influencing the success of aquatic Hyphomycetes at this site.

At sites A to F, the overall abundance of aquatic Hyphomycetes, and the number of species found, was much reduced compared to the stream site. The contrast was particularly marked between the sporas revealed by filtration.

Probably the most important factor influencing the number of aquatic Hyphomycetes found at the tributary sites was the relatively small amount of leaf material entering a relatively large volume of water.

The flora and spora of Site A was also affected by dirt covered leaves.

The relatively huge volume of water receiving imported spores from tributaries and via runoff provides the most likely explanation for the sparse sporas found at the main river sites.

Three important species were recorded from all six of the river sites and can perhaps be regarded as characteristic of this part of the River Wear 'system'. These were T.marchalianum (flora and spora), A.longissima (flora) and H.lugdunensis (spora).

In comparing the variation in abundance and species of aquatic Hyphomycete between sampling dates, no simple pattern emerged either within or between the sites studied.

Certainly no seasonal pattern was expected, or emerged. A plausible explanation for the variations, based on increase in both flow and spore production due to rain,was put forward. The balance between these two, resulting in a certain density of spores per unit volume could not be predicted, however,&the relationship proposed between amount and rainfall and observed differences may be entirely spurious.

Certain differences were expected between the sporas and floras revealed by the various methods, and although differences did occur, they were not always particularly clear cut.

Obviously the species and abundance of spores revealed by the filter and foam methods differed greatly from the floras revealed by leaf disc observation methods, since a completely different aggregate of species was being sampled.

In respect of the species found, foam appeared to the more informative for a site with a low concentration of spores, whilst the filter sample appeared to be more representative for sites with a higher concentration.

The shorter water incubations did appear to back up observations made on unincubated material. However the 1SA incubations were rather different. Smaller numbers of aquatic Hyphomycete species were recorded, even on leaf material from the 3 day 1SA incubation. The ability of the longer incubations to reveal the inactive and largely terrestrial 'flora' of the leaves was demonstrated quite well, however.

5.3.2 Practical and Theoretical conclusions

5.3.2.1 Constraints on ecological investigations

Carrying out the present field study has led me to certain conclusions about the pursuit of ecological research; some of the ideas explored are discussed briefly below.

It appears that various constraints are experienced in the planning and execution of an ecological investigation. There are two main types of constraint: those you can do little or nothing about, and those you may be able to do something about. Both these types of constraint have been discussed in Chapters 4 and 5, in the introduction to and discussion of the field study programme.

Good examples of the first type of constraint are: the time and money available (general to research); the necessity for sporulation in the identification of fungi; (general to mycological research); the necessity of simulating environmental conditions(in fungal ecology); and the availability of suitable leaf material (this study).

Overcoming the second type of constraint depends on the use of good ecological understanding and technique, that is,a combination of technical expertise and a good background in both the theoretical and practical aspects of a proposed study. Obviously knowledge without expertise and expertise without knowledge are not as powerful as the combination. The first is inevitable in a student new to ecological investigation; the second should be avoided at all costs.

Ecological understanding is useful in appreciating the 'true' or 'actual' situation,as illustrated in Fig. 5.2,whilst practical knowledge and expertise is useful in appreciating those factors which influence the 'revealed' or 'perceived' situation, also illustrated in Fig.5.2.

Variation is the hall mark of things biological. Some variations are of interest to the ecological researcher, and are selected for investigation:

for example the differences in aquatic Hyphomycete spora displayed by lotic sites of different character. However unwanted variation due to a variety of sources, both known and unknown,will also occur.

These variations are good examples of the second type of constraint. To release the constraint 'unwanted' variation must, as far as possible, either be removed or taken intoaccount. Thus in the present investigation only one species of leaf was collected in order to study the aquatic Hyphomycete flora of a particular site, and some unnecessary variation was avoided. On the other hand the leaf material collected was not of uniform condition, and since a comparison between thick and skeletonized tissue, for example, was not envisaged, the variation it produced in the flora recorded was unwanted.

In a laboratory study some of the variation can be controlled. For instance, the temperature of incubation can be kept constant. In a field study this is not possible.

Statistics appears to be a powerful tool in overcoming this second type of constraint. Used (in a co-ordinated fashion) at each of the three 'stages' of an ecological investigation-field sampling, lab. processing and data analysis - it can overcome unwanted variation.

For example, if more leaves had been available at each site, a random sub-sample could have been taken from a larger sample of leaves, and the material then available for observation and incubation would have been more represenative. At the analysis stage of the investigation the use of an appropriate statistical method of comparison would have distinguished between real and apparent differences in the data being assessed.

5.3.2.2 Alternatives to the present programme

The experience of carrying out the present programme revealed numerous possible 'improvements' that could be incorporated at every stage of planning, sampling and processing.

For instance, larger volumes of water filtered would give a better picture of the aquatic Hyphomycete spora of a particular site, whilst a larger number of randomly selected leaf discs observed would provide a more accurate picture of the flora. More rigorous comparisons between floras and sporas would be possible with the more detailed data thus obtained.

All these improvements, however, would make it impossible for the present study to be carried out on the same time scale. However, several "condensed" programmes arise naturally from the original investigation, with its three main areas of comparison.

It would be possible, using improved methods of sampling, processing and assessment, to produce an interesting three-month study on one of the following:

A comparison of the flora and spora at a single site; a comparison of the flora of different species of leaf at a single site; a comparison of the flora of one species of leaf from a number of sites; a comparison of the sporas of a number of sites, etc, etc.

It would also be possible, with such a tailored study, to fit in a certain amount of preliminary work, allowing some of the sources of practical difficulty and biological variation to be identified and taken into account.

Although all of these small studies would provide interesting conclusions on aquatic Hyphomycetes in the Durham area they would not advance considerably the understanding of the ecology of this group of fungi as a whole. There is obviously great scope for laboratory work on the nutritional capabilities of the more common and well known species of aquatic Hyphomycete. The conclusions from such a study would be most helpful in increasing our understanding of the significance of aquatic Hyphomycetes as decomposers in the aquatic ecosystem.

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TABLE 5.9

Most common species; maximum and minimum abundance

	Site A	Site B	Site C	Site D	Site E	Site F	Site G
FILTER Most common species Maximum A & D Minimum A & D	unknown 'A' 10:8:77 1&8:9:77 N.R.	Tetracladium marchalianum 24.8.77 10&18:8:77 N.R.	Tetracladium marchalianum 10:8:77 18:8:77 N.R.	Tetracladium marchalianum 18:8:77 8:9:77	Tetracladium marchalianum 24:8:77 10:8:77	Tetracladium marchalianum 1:9:77 10&18:8:77 N.R.	Articulospora tetracladia 10:8:77 24:8:77
FOAM Most common species Maximum A & D Minimum A & D				(Entomopthora) Tetracladium marchalianum 24:8:77 8:9:77			C.stellata splendens 21:8:77 8:9:77
DIRECT OBSERVATION	Tetracladium marchalianum		Tetracladium marchalianum	Tetracladium marchalianum	Tetracladium marchalianum		Tricladium splendens
WATER INCUBATION	Tetracladium marchalianum		Anguillospora	T.angulatum T.marchalianum	Anguillospora		Tricladium splendens
ISA INCUBATION	Tetracladium marchalianum		Alternaria	Tetrocladium marchalianum	Tetracladium marchalianum		T.terrestre
PLATE	Fusarium		Fusarium	T.setigerum T.marchalianum	Fusarium		T.terrestre Fusarium

A = abundance D = diversity N.R. = no spores recorded

TABLE 5.8

Aquatic Hyphomycete Species Recorded by all Methods

	A	B	C	D	E	F	G
Tricladium splendens				*			* + 0 o
Tricladium angulatum				+ 0 o			*
Tricladium terrestre					*		* + 0 o
Articulospora tetracladia				+			* + 0
Lemmoniera aquatica							*
Lemmoniera terrestris							* +
Alatospora acuminata							* + 0
Tetrachaetum elegans							* +
Tetracladium marchalianum	* 0	*	* 0 o	* + 0 o	* 0	*	* 0
Tetracladium setigerum				* 0 o			*
Clavariopsis aquatica		*	0	+	* 0	*	* 0
Flagellospora curvula			*	*	*	*	* + 0
Anguillospora longissima	* 0	*	* 0	+ 0	* 0	*	* +
Centrospora acerina							*
Camposporium pellucidum							* + 0
Heliscus lugdunensis	*	*	*	* +	*	*	* +
Clavatospora stellata							* +
Dactylella aquatica		*		+			* 0
(Entomopthora sp.)				* +	*		*
Tripospermum myrti	*						
Volucrispora sp.					*		*
Flabellospora sp.			o				
Anguillospora crassa				+			+
Unknown A	*	*		*	*		*
Unknown B	*						
Unknown C	*				*		
Unknown D					*		
('K' spore)					*		
('H' spore)		*				*	
TOTALS	7 2	7	4 3 1	7 9 3 3	12 3	6	21 12 9 2

KEY
* Filter : spores
+ Foam : spores
0 Leaf discs
o Plate (ISA) attached spore only

Details of records from leaf discs can be found in Tables 5.7 (summary) 5.6 (all data)

Details of foam and filter in Table 5.5

Extra Notes

Unknown A	= unknown A	pg 525	Ingold	1975c
Unknown B	= unknown no.5	pg 91	Ingold	1975a
Unknown C	= unknown no.2	pg 91	Ingold	1975a
Unknown D	= unknown no.11	pg 91	Ingold	1975a

'K' spore
'H' spore see photographs

TABLE 5.7

Summary of Leaf Disc Data

for attached spores only

A species name underlined = observed growing on plate only
A species name ticked = observed on plate and disc

Method	Site A	Site C	Site D	Site E	Site G
Direct Observation	T.marchalianum	T.marchalianum C.aquatica	T.marchalianum T.setigerum T.angulatum	T.marchalianum C.aquatica	T.splendens A.tetracladia F.curvula
Water Incubation 3 days	T.marchalianum A.longissima	Anguillospora sp.	T.marchalianum T.setigerum T.angulatum A.longissima	T.marchalianum A.longissima C.aquatica	T.marchalianum C.aquatica T.splendens A.tetracladia F.curvula D.aquatica
7 & 10 days	A.longissima	T.marchalianum Anguillospora	T.marchalianum T.setigerum T.angulatum	T.marchalianum T.angulatum C.aquatica	T.splendens F.curvula A.acuminata
ISA Incubation 3 days	T.marchalianum Fusarium	Alternaria ✓ T.marchalianum Fusarium Aureobasidium Cladosporium	T.marchalianum T.setigerum Cladosporium	T.marchalianum Fusarium Cladosporium	T.splendens T.terrestre ✓ A.tetracladia C.pellucidum Fusarium ✓ Alternaria Aureobasidium Cladosporium
7 & 10 days	A.longissima Fusarium	Fusarium Alternaria Cladosporium Elabellospora	T.marchalianum ✓ T.setigerum ✓ T.angulatum Fusarium Alternaria Cladosporium Mucor	T.marchalianum Fusarium Aureobasidium	T.splendens ✓ T.terrestre ✓ Fusarium ✓ Alternaria ✓ Cladosporium ✓ Aureobasidium Geotrichum Penicillium

TABLE 2.6

Aquatic Hyphomycete

Floras and Sporas:

dominant species;

seasonal and successional records;

methods used.

KEY

C	constant species
C(W)	constant: particularly common in winter
C(S)	constant: particularly common in summer
W/S	winter species: more common in spring
W/A	winter species: more common in autumn
S	summer species
+	species particularly associated with the tropics
o	on introduced leaf material (old)
n	on naturally occurring leaves (new)

TABLE 2.6

← Temperate Rivers and Streams →

AUTHOR	Nilsson 1964	Conway 1970	Triska 1970	Ingold 1973
LOCATION	Various streams in Sweden	Streams New York State	Woodland stream (Pittsburgh) U.S.A.	Various streams in Britain
METHODS	various	Flora: leaves taken from stream	Flora: Introduced leaves and naturally occurring leaves	Spora: Foam and scum
	dominant Swedish species: A.acuminata A.longissima A.tetracladia C.aquatica F.curvula L.aquatica T.splendens T.marchalianum V.elodeae	"ever-present" species: A.acuminata C A.longissima C C.aquatica C L.aquatica C T.marchalianum C	A.acuminata* C(W) A.tetracladia C(S) L.aquatica* C T.elegans So T.marchalianum So T.monosporus +So C.longibrachiata Sn C.stellata Sn *particularly dominant	extremely common: C.aquatica L.aquatica T.marchalianum

Continued...

TABLE 2.6 (Continued)

← Temperate Rivers and Streams →

Willoughby & Archer 1973	Iqbal & Webster 1973	Suberkropp & Klug 1974	Barlocher & Kendrick 1974
Wooded stream Lake District, UK.	River system: River Exe, Devon	Woodland stream Michigan U.S.A.	Wooded stream Ontario, Canada
Spora: Foam and scum Flora: from twigs	Spora: Quantitative filtration method	Flora: in situ: scanning electron microscopy	Flora: Introduced sterile and non-sterile leaves
Most common: H.lugdunensis C L.aquatica C T.marchalianum C T.splendens C	Most common: (A.acuminata) C A.longissima C (A.tetracladia) C T.marchalianum C.aquatica W/S C.longibrachiata W/S F.curvula W/A L.curvula +S ( ) slightly less common	The dominant winter and summer species: A.acuminata W L.curvula +S	Most common: A.acuminata A.longissima H.lugdunensis L.aquatica T.angulatum T.marchalianum

Continued...

TABLE 2.6 (Continued)

Temperate Rivers and Streams		Tropical Stream	Terrestrial Records
Linsey & Glover 1975	Suberkropp & Klug 1976	Padgett 1977	Koske & Duncan 1974
Wooded stream Lincolnshire, UK.	Wooded stream Michigan, USA	Rain forest stream Puerto Rico	Woodland British Columbia
Spora: Foam and impaction trap	Flora: Introduced leaves	Spora: foam Flora: introduced and naturally occurring leaves	Flora: Litter leaves
Most common: C.aquatica C(W) F.curvula C T.marchalianum C(S) A.acuminata W/A L.aquatica W	Dominant species: A.acuminata Anguillospora C.aquatica F.curvula T.marchalianum	Dominant species: Campylospora chaetocladia + Pyrimidospora casuarina + L.curvula + T.monosporus +	Common species: A.tetracladia C(W) T.splendens C(W) V.elodeae C(W) A.acuminata W G.craginiformis W T.setigerum W V.graminae W

TABLE 5.1

pH of water: measured c. 2 hours after collection

Date	Site: A	B	C	D	E	F	G
3:8:77	8.5	7.8	8.5	8.5	8.4	8.5	7.5
10:8:77	8.4	7.8	8.5	8.4	8.5	8.7	7.5
18:8:77	8.6	8.1	8.2	8.4	8.4	8.4	7.5
24:8:77	8.7	8.0	8.5	8.6	8.4	8.4	7.5
1:9:77	8.7	8.1	8.6	8.7	8.4	8.3	7.5
8:9:77	8.8	8.7	8.7	8.9	8.8	8.5	7.5
Mean	8.6	8.0	8.5	8.5	8.5	8.5	7.5

TABLE 5.2

Temperature of water (^{o}C) measured between 09.30-11.30

Date	Site: A	B	C	D	E	F	G
3:8:77	11.5	13.0	13.0	12.0	13.0	13.5	-
10:8:77	9.0	11.0	10.5	10.0	11.0	12.0	9.0
18:8:77	10.0	11.0	11.0	10.0	11.0	12.0	9.0
24:8:77	9.0	10.5	10.0	9.0	10.5	12.0	8.0
1:9:77	9.0	9.5	9.5	9.5	10.5	10.0	8.0
8:9:77	6.5	8.5	8.0	7.5	8.5	9.0	7.0

TABLE 5.3

River Authority Data for Sites A-F

(12 month averages for 1976)

Sample Sites:	pH	Total Solids	Nitrogen as N			Hardness as $CaCO_3$ (total)	B.O.D. 5 days at 20°C	Dissolved O_2 % saturation	Phosphate as P
			Ammoniacal	Nitrous	Nitric				
A Croxdale Beck (R.A.38)	8.3	1520	0.20	0.12	8.10	447	3.0	91	1.26
B River Wear (R.A.34)	7.6	415	0.13	0.04	2.10	219	2.1	95	0.32
C River Browney (R.A.37)	7.9	478	1.14	0.67	4.69	255	3.8	89	0.73
D River Deerness (R.A.35)	7.8	631	0.19	0.04	3.30	397	2.1	102	0.27
E River Browney (R.A.36)	7.9	425	0.16	0.05	4.83	216	2.4	94	0.55
F River Wear (R.A.39)	7.9	463	0.17	0.04	2.60	230	2.5	99	0.32
		ppm	ppm	ppm	ppm	ppm	mg/l	%	ppm

(R.A.38) = River Authority sampling point number: see Map 4.1

TABLE 5.4

Rainfall Data

Readings at Durham University Observatory

Date		Rainfall	Sampling Days
Aug.	1	0.1 mm	
	2	Tr	
	3	-	- (Preliminary samples)
	4	0.2 mm	
	5	-	
	6	-	
	7	-	
	8	-	
	9	-	
	10	-	- 1st main sample
	11	-	
	12	Tr	
	13	0.8 mm	
	14	0.3 mm	
	15	Tr	
	16	-	
	17	4.5 mm	
	18	2.2 mm	- 2nd main sample
	19	10.2 mm	
	20	0.3 mm	
	21	1.7 mm	- Extra sample: Site G
	22	-	
	23	Tr	
	24	6.2 mm	- 3rd main sample
	25	2.2 mm	
	26	6.1 mm	
	27	Tr	
	28	-	
	29	2.1 mm	
	30	0.9 mm	
	31	Tr	
Sept.	1	0.3 mm	- 4th main sample
	2	-	
	3	-	
	4	-	
	5	2.5 mm	
	6	2.0 mm	
	7	-	
	8	-	- 5th main sample

TABLE 5.5

(5.5a → 5.5g)

FILTER AND FOAM DATA

SITES A → G

DATES: 10:8:77 → 8:9:77

(extra collection 28:7:77, site G)

Abbreviations

T.march.	Tetracladium marchalianum
H.lugd.	Heliscus lugdunensis
C.stell.	Clavatospora stellata
A.tetra.	Articulospora tetracladia
T.splend.	Tricladium Splendens
A.long.	Anguillospora longissima
F.curv.	Flagellospora curvula
A.acum.	Alatospora acuminata
Ent.	Entomophthora sp.
Unk.A.	Unknown A
Unk.B.	Unknown B

Filter spore totals

Totals are given as spores/400 ml Except site G where they are /100 ml. The actual volume of water filtered to produce each site's spore records is given in brackets above each column.

For further details

see text, Table 5.8 and Table 5.9.

TABLE 5.5a

SITE A: CROXDALE BECK

	10.8.77 Filter (50)	18.8.77 Filter (2x25)	24.8.77 Filter (2x25)	1.9.77 Filter (2x25)	8.9.77 Filter (2x25)
Tricladium splendens				sample taken but no spores found abundant non-living particles	sample taken but no spores found abundant non-living particles
Tricladium angulatum					
Tricladium terrestre					
Articulospora tetracladia					
Lemmoniera aquatica					
Lemmoniera terrestris					
Alatospora acuminata					
Tetrachaetum elegans					
Tetracladium marchalianum					
Tetracladium setigerum	8				
Clavariopsis aquatica					
Flagellospora curvula					
Anguillospora longissima	8				
Centrospora acerina					
Camposporium pellucidum					
Heliscus lugdunensis	16				
Clavatospora stellata					
Dactylella aquatica					
(Entomopthora sp.)					
Tripospermum myrti		8			
Volucrispora sp.					
Flabellospora sp.					
Anguillospora crassa					
Unknown A	8	24			
Unknown B			8		
Unknown C		8			
Unknown D					
('K' spore)					
('H' spore)					
Total spore numbers	40	40	8		
Number of species	4	3	1		
Common species	H.Lugd.	Unk.A.	Unk.B.		

TABLE 5.5b

SITE B: RIVER WEAR

	10.8.77 Filter (100)	18.8.77 Filter (2x50)	24.8.77 Filter (2x50)	1.9.77 Filter (2x50)	8.9.77 Filter (2x50)
Tricladium splendens	Sample taken but no spores found Abundant algae	Sample taken but no spores found Abundant algae			
Tricladium angulatum					
Tricladium terrestre					
Articulospora tetracladia					
Lemmoniera aquatica					
Lemmoniera terrestris					
Alatospora acuminata					
Tetrachaetum elegans					
Tetracladium marchalianum			12	12	8
Tetracladium setigerum					
Clavariopsis aquatica			4		8
Flagellospora curvula					8
Anguillospora longissima					
Centrospora acerina					
Camposporium pellucidum					
Heliscus lugdunensis			4		
Clavatospora stellata					
Dactylella aquatica					
(Entomopthora sp.)					
Tripospermum myrti					
Volucrispora sp.					
Flabellospora sp.					
Anguillospora crassa					
Unknown A			8		
Unknown B					
Unknown C					
Unknown D					
('K' spore)					
('H' spore)			12		
Total spore numbers			40	12	24
Number of species			5	1	3
Common species			T.march. 'H'	T.march.	T.march. C.aquat. F.curv.

TABLE 5.5c

SITE C: RIVER BROWNEY

	10.8.77 Filter (2x100)	18.8.77 Filter (2x100)	24.8.77 Filter (2x100)	1.9.77 Filter (100)	8.9.77 Filter (100)
Tricladium splendens		Sample taken but no spores found Abundant algae			
Tricladium angulatum					
Tricladium terrestre					
Articulospora tetracladia					
Lemmoniera aquatica					
Lemmoniera terrestris					
Alatospora acuminata					
Tetrachaetum elegans					
Tetracladium marchalianum	12		6	28	16
Tetracladium setigerum					
Clavariopsis aquatica					
Flagellospora curvula					8
Anguillospora longissima	2		2		4
Centrospora acerina					
Camposporium pellucidum					
Heliscus lugdunensis	2		4		
Clavatospora stellata					
Dactylella aquatica					
(Entomopthora sp.)	2				
Tripospermum myrti					
Volucrispora sp.					
Flabellospora sp.					
Anguillospora crassa					
Unknown A					
Unknown B					
Unknown C					
Unknown D					
('K' spore)					
('H' spore)					
Total spore numbers	18		12	28	28
Number of species	4		3	1	3
Common species	T.march.		T.march.	T.march.	T.march

TABLE 5.5d

SITE D: RIVER DEERNESS

	10.8.77		18.8.77	24.8.77		1.9.77		8.9.77	
	Filter (2x200)	Foam	Filter (200)	Filter (200)	Foam	Filter (200)	Foam	Filter (200)	Foam
Tricladium splendens			2						
Tricladium angulatum					8		1		
Tricladium terrestre									
Articulospora tetracladia		4							2
Lemmoniera aquatica									
Lemmoniera terrestris									
Alatospora acuminata		3							
Tetrachaetum elegans									
Tetracladium marchalianum	5	12	80	10	7	6	1		12
Tetracladium setigerum			6						
Clavariopsis aquatica					1				
Flagellospora curvula			2						
Anguillospora longissima		2			23		1		8
Centrospora acerina									
Camposporium pellucidum									
Heliscus lugdunensis	4	3			4		4	2	4
Clavatospora stellata									
Dactylella aquatica					1				
(Entomopthora sp.)		13	16		30	2			2
Tripospermum myrti									
Volucrispora sp.									
Flabellospora sp.									
Anguillospora crassa									
Unknown A	3								
Unknown B									
Unknown C									
Unknown D									
('K' spore)									
('H' spore)									
Total spore numbers	12	37	106	10	74	8	7	2	28
Number of species	3	6	5	1	7	2	4	1	5
Common species	T.march.	T.march. (Ent)	T.march.	T.march.	A.long. (Ent)	T.march.	H.lugd.	H.lugd.	T.march

TABLE 5.5e

SITE E: RIVER BROWNEY

	10.8.77 Filter (100)	18.8.77 Filter (50)	24.8.77 Filter (2x50)	1.9.77 Filter (2x50)	8.9.77 Filter (50)
Tricladium splendens					
Tricladium angulatum				4	
Tricladium terrestre		8			
Articulospora tetracladia					
Lemmoniera aquatica					
Lemmoniera terrestris					
Alatospora acuminata					
Tetrachaetum elegans					
Tetracladium marchalianum	4	40	76	80	24
Tetracladium setigerum					
Clavariopsis aquatica		8	8	16	
Flagellospora curvula			4	8	8
Anguillospora longissima	8		8	4	8
Centrospora acerina					
Camposporium pellucidum					
Heliscus lugdunensis	4				
Clavatospora stellata					
Dactylella aquatica					
(Entomopthora sp.)				4	
Tripospermum myrti					
Volucrispora sp.			4		
Flabellospora sp.					
Anguillospora crassa					
Unknown A			8		
Unknown B					
Unknown C					
Unknown D			4		
('K' spore)					16
('H' spore)					
Total spore numbers	16	56	112	116	50
Number of species	3	3	7	6	4
Common species	A.long	T.march.	T.march.	T.march.	T.march

TABLE 5.5f

SITE F: RIVER F

	10.8.77 Filter (100)	18.8.77 Filter (100)	24.8.77 Filter (100)	1.9.77 Filter (50)	8.9.77 Filter (2x50)
Tricladium splendens	Sample taken but no spores found Abundant	Sample taken but no spores found			
Tricladium angulatum					
Tricladium terrestre					
Articulospora tetracladia					
Lemmoniera aquatica					
Lemmoniera terrestris					
Alatospora acuminata					
Tetrachaetum elegans					
Tetracladium marchalianum			8	16	4
Tetracladium setigerum					
Clavariopsis aquatica				8	4
Flagellospora curvula					8
Anguillospora longissima				8	
Centrospora acerina					
Camposporium pellucidum					
Heliscus lugdunensis			4	8	
Clavatospora stellata					
Dactylella aquatica					
(Entomopthora sp.)					
Tripospermum myrti					
Volucrispora sp.					
Flabellospora sp.					
Anguillospora crassa					
Unknown A					
Unknown B					
Unknown C					
Unknown D					
('K' spore)					
('H' spore)			4		
Total spore numbers			16	40	16
Number of species			3	4	3
Common species			T.march.	T.march.	F.curv.

TABLE 5.5g
SITE G: WOODLAND STREAM

	28.7.77		10.8.77		18.8.77	21.8.77		24.8.77	1.9.77	8.9.77	
	Filter (100)	Foam	Filter (50)	Scum	Filter (2x50)	Filter (100)	Foam	Filter (100)	Filter (100)	Filter (100)	Scum
Tricladium splendens	64	13	158	102	2	27	11	2	9	42	23
Tricladium angulatum	1		6			1				1	
Tricladium terrestre	5		40	2	2				1	5	1
Articulospora tetracladia	45	7	218	17	5	45	14	5	15	103*	17
Lemmoniera aquatica	18	4	6		1	16	1			1	
Lemmoniera terrestris	1	1	6	3		2				2	
Alatospora acuminata	16	13	40	1		11	59		1	17	13
Tetrachaetum elegans	5		6	1		2		1	4	1	1
Tetracladium marchalianum	5		10						1	3	1
Tetracladium setigerum	2		22				1				
Clavariopsis aquatica	1					2	1			1	1
Flagellospora curvula	16	3	56	6	18	20	9	7	9	65	5
Anguillospora longissima	1		16	9	1	3					2
Centrospora acerina	2	1	4							3	2
Camposporium pellucidum	2	2	46	20						1	
Heliscus lugdunensis	91	19	70	8	1	23	39	3	3	3	1
Clavatospora stellata	44	20	104	5		34	63	5	3	2	5
Dactylella aquatica	3	1				20	20	1	1	1	
(Entomopthora sp.)			8			1	2			24	
Tripospermum myrti											
Volucrispora sp.						1					
Flabellospora sp.											
Anguillospora crassa				2							
Unknown A			8	3							
Unknown B											
Unknown C											
Unknown D											
('K' spore)											
('H' spore)											
Total spore numbers	326	84	828	179	30	208	219	24	47	275	72
Number of species	18	11	18	13	7	15	17	7	10	17	12
Common species	H.lugd. T.splend. C.stell. A.tetra	C.stell. H.lug.	A.tetra. T.splend. C.stell.	T.splend.	F.curv.	A.tetra. C.stell	C.stell. A.acum.	F.curv.	A.tetra	*A.tetra.	T.splend

*A.tetra f. tetra 50
*A.tetra f. ang. 53

TABLE 5.6

(5.6a, c, d, e, g)

LEAF DISC DATA

SITES: A, C, D, E, G

DATES: 24 : 8 : 77
9 : 9 : 77

Abbreviations:

D.O.	=	records from Direct Observation of leaf discs
H_2O	=	records from water incubated leaf discs
ISA	=	records from ISA incubated leaf discs
P	=	records from surface of medium (plate)
(3),(7),(10)	=	3, 7 or 10 days incubation
D1,D2...	=	1st, 2nd ... leaf disc scanned
0,1,2,3	=	density scale see Table 4.5

TABLE 5.6a SITE A: Croxdale Beck

	Species	Date of sample 28:8:77					9:9:77				
		Density Scores D1	D2	D3	D4	D5	D1	D2	D3	D4	D5
D.O.	T.marchalianum	0	0	1	2	1	Nothing observed				
	Fusarium	0	0	0	0						
H_2O	T.marchalianum	0	0	1	0	0	1	0			
(3)	A.longissima					0	1	0			
	Fusarium			0							
(7)	T.marchalianum	0		0			0				
	A.longissima						1	1			
(10)	T.marchalianum		0					0			
	Lemmoniera sp.	0	0								
	T.angulatum	0									
	Fusarium						0	0	0	0	
ISA	T.marchalianum	0	0				0	0	1	1	
(3)	Fusarium	0	0								
(7)	T.marchalianum	0	0				0	0	0		
	A.longissima						1				
(10)	———						Nothing observed				
P											
(3)	T.marchalianum		0						0		
	A.longissima								0		
	Fusarium		2						1		
(7)	T.marchalianum		0								
	A.longissima			much bacterial growth					0	much bacterial growth	
	Fusarium		2								
(10)	T.marchalianum	Erosion							0	much bacterial growth	

TABLE 5.6c SITE C: River Browney (A1)

	Species	Date of sample 28:8:77 Density Scores D1	D2	D3	D4	D5	9:9:77 D1	D2	D3	D4	D5
D.O.	T.marchalianum	1	2	3			Nothing observed				
	L.aquatica	0									
	C.aquatica		1								
H_2O	T.marchalianum			0	0						
	Anguillospora sp.	2	2	2	2	1	1				
(3)	Lemmoniera sp.		0								
	C.aquatica		0	0	1						
	T.angulatum		1								
(7)	T.marchalianum	1									
	Anguillospora sp.	1	1								
	Lemmoniera sp.	0					0				
(10)	Lemmoniera sp.	Nothing observed bacteria					0	0	0		
ISA	T.marchalianum	0	0						0		
(3)	Lemmoniera sp.	0									
	Alternaria						1	1	1		
(7)	T.marchalianum	0						0			
	H.lugdunensis	0					0	0	0		
	Flabellospora sp.						0	0	0		
	Alternaria		0								
(10)		Nothing observed					Nothing observed				
P	T.marchalianum			0					2		
(3)	Fusarium			2					2		
	Aureobasidum								2		
	Alternaria								1		
	Cladosporium								1		
(7)	T.marchalianum			0					0		
	Flabellospora sp.								2		
	Fusarium								1		
	Alternaria								1		
	Cladosporium								1		
(10)	T.marchalianum								0		
	Fusarium			bacterial					2		
	Aureobasidium			erosion					1		
	Cladosporium								1		

TABLE 5.6d SITE D: River Deerness

	Species	Date of sample 28:8:77					9:9:77				
		Density Scores									
		D1	D2	D3	D4	D5	D1	D2	D3	D4	D5
D.O.	T.marchalianum	1	1	1			2	2			
	Anguillospora sp.	0									
	T.setigerum		1								
	T.angulatum	2	2								
H_2O (3)	T.marchalianum	1	0	0	1		0	0	2	0	1
	Anguillospora sp.		1								
	T.setigerum				1						
	T.angulatum	0	0		1						1
(7)	T.marchalianum	1					2	0	1	0	0
	T.angulatum	1	2	3	0	0		1	1		1
(10)	T.marchalianum						0	1	1	2	
	T.setigerum	1			0						
	T.angulatum	1	2	3	0						
ISA (3)	T.marchalianum	0	0		0	0	0	0	2	0	
	T.setigerum			1							
	T.angulatum			0							
(7)	T.marchalianum	0	1	0	0	0	0	3	3	0	0
	T.setigerum		1	0	0	0					
	T.angulatum			0	0	0					
(10)	T.marchalianum	0	0		0	0	1	0	0	0	
	T.setigerum	0	0			0					
	T.angulatum		1	1							
P (3)	T.marchalianum			0					0		
	T.angulatum			0							
	Cladosporum								1		
(7)	T.marchalianum			1					1		
	T.setigerum			3							
	T.angulatum			1							
	Fusarium			3							
	Aureobasidium								1		
	Alternaria								1		
(10)	T.marchalianum			1					0		
	T.setigerum			2	erosion						
	Cladosporium								1		
	Mucor								1		

TABLE 5.6e SITE E: River Browney

	Species	Date of sample 28:8:77 Density Scores D1	D2	D3	D4	D5	Date of sample 9:9:77 Density Scores D1	D2	D3	D4	D5
D.O.	T.marchalianum	3	2	1					0		
	Lemmoniera								0		
	C.aquatica						0	1	0		
H_2O (3)	T.marchalianum	1	1	0					0		
	Anguillospora			1			2			1	
	C.aquatica			1			2	0		0	0
	Botrytis								1		
(7)	T.angulatum	1					0				
	C.aquatica	1							algae		
(10)	T.marchalianum	1	0	0			Nothing observed			algae	
ISA (3)	T.marchalianum	1	1	1	1		Nothing observed			algae	
		2	2								
(7)	T.marchalianum	3	3				0	0			
	Fusarium	0	0				0	0	0	0	
(10)	T.marchalianum		0	2			Nothing observed			bacteria	
	Fusarium	0									
P (3)	T.marchalianum			0					0		
	Fusarium			2					1		
	Cladosporium								1		
(7)	T.marchalianum			0					0		
	T.angulatum								0		
	Fusarium			1					1		
	Aureobasidium								2		
	Geotrichum								1		
(10)	T.marchalianum			1					0		
	Fusarium			1	erosion					erosion	

TABLE 5.6g SITE G: Woodland stream

	Species	Date of sample 28:8:77 Density Scores D1	D2	D3	D4	D5	Date of sample 9:9:77 Density Scores D1	D2	D3	D4	D5
D.O.	T.splendens	2					2	2	2	1	1
	A.tetracladia	1					1	1	1		
	F.curvula	1					1	1	1		
H_2O	T.marchalianum			1							
(3)	T.splendens	2	1	3	2		2	2	2	1	1
	A.tetracladia				1		1	1	1		
	F.curvula				1						
	C.aquatica				1						
	D.aquatica			1	2						
(7)	T.splendens	0	1				2	1	1	2	
	A.tetracladia						1				
	F.curvula						0	1			
	C.aquatica		0								
	A.acuminata								1		
	L.terrestris		0								
(10)	T.splendens	2	2				Nothing observed				
	A.tetracladia		0								
	F.curvula	1	0	1	1						
ISA	T.splendens	0	1					0			
(3)	A.tetracladia						1				
	T.terrestre						3	2	2	0	
	C.pellucidum						0			1	
	Fusarium	0	0							2	
	Alternaria	0								1	
(7)	T.splendens	Nothing observed					0		1		
	T.terrestre						2	2	1	1	2
	Fusarium							1		3	2
	Alternaria								0	1	1
	Cladosporium										1
(10)	T.splendens							1	0		
	T.terrestre						2	2	1	2	
	Fusarium	0	0						2	2	1
	Alternaria			1							
	Cladosporium								1	1	1

Continued...

TABLE 5.6g (Continued)

		D1	D2	D3	D4	D5	D1	D2	D3	D4	D5
P	T.splendens			0							
(3)	T.terrestre								2		
	Fusarium			2					2		
	Aureobasidium			1					1		
	Cladosporium								1		
	Geotrichum			2					1		
(7)	T.splendens			0					1		
	T.terrestre								2		
	C.pellucidum								0		
	Fusarium				erosion				2		
	Alternaria								1		
	Aureobasidium			1					1		
	Geotrichum								1		
	Penicillium								1		
(10)	T.terrestre								2		
	C.pellucidum								0		
	Fusarium			erosion					2	erosion	
	Cladosporium								1		

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READ (5,17) (X(JR),JR=JST,JE)                                   00005300
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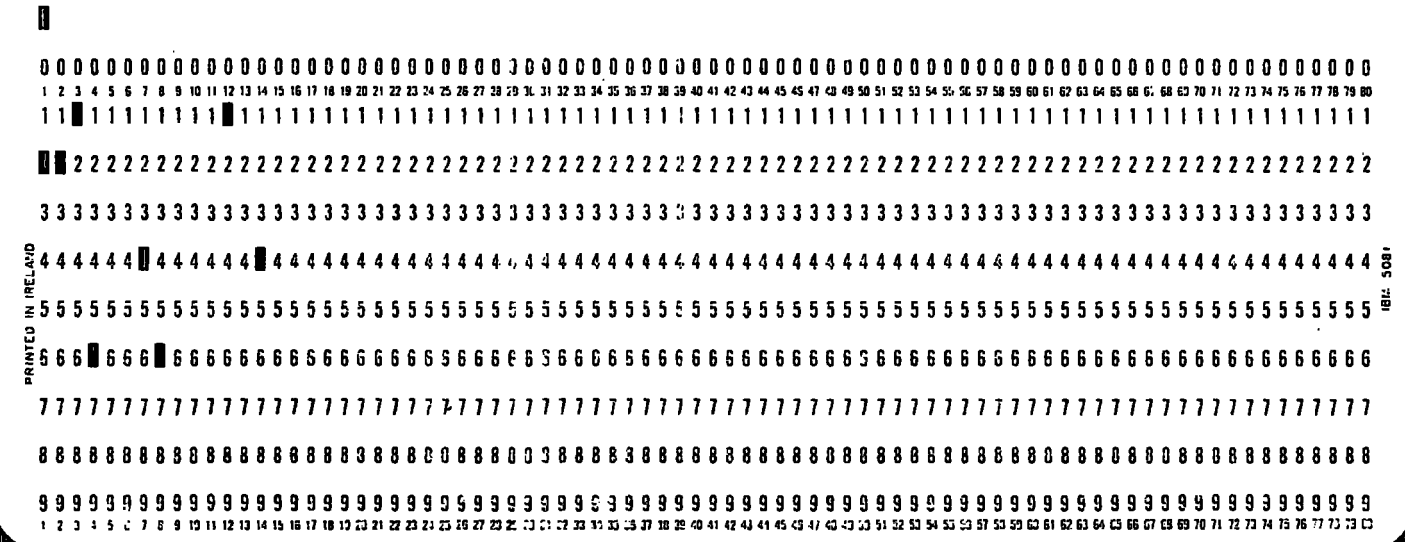

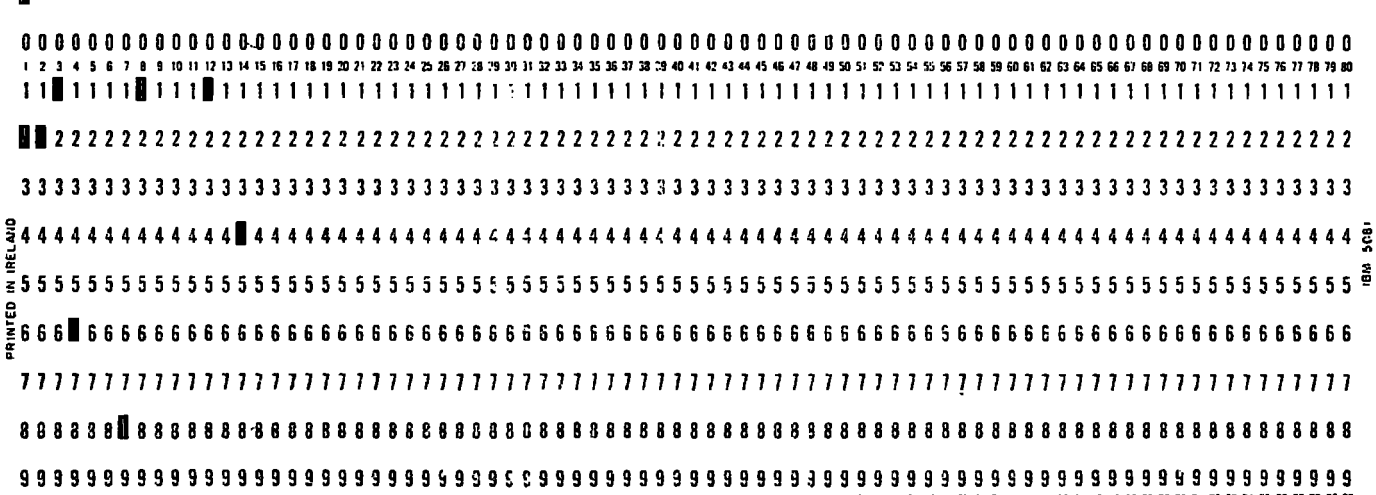

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