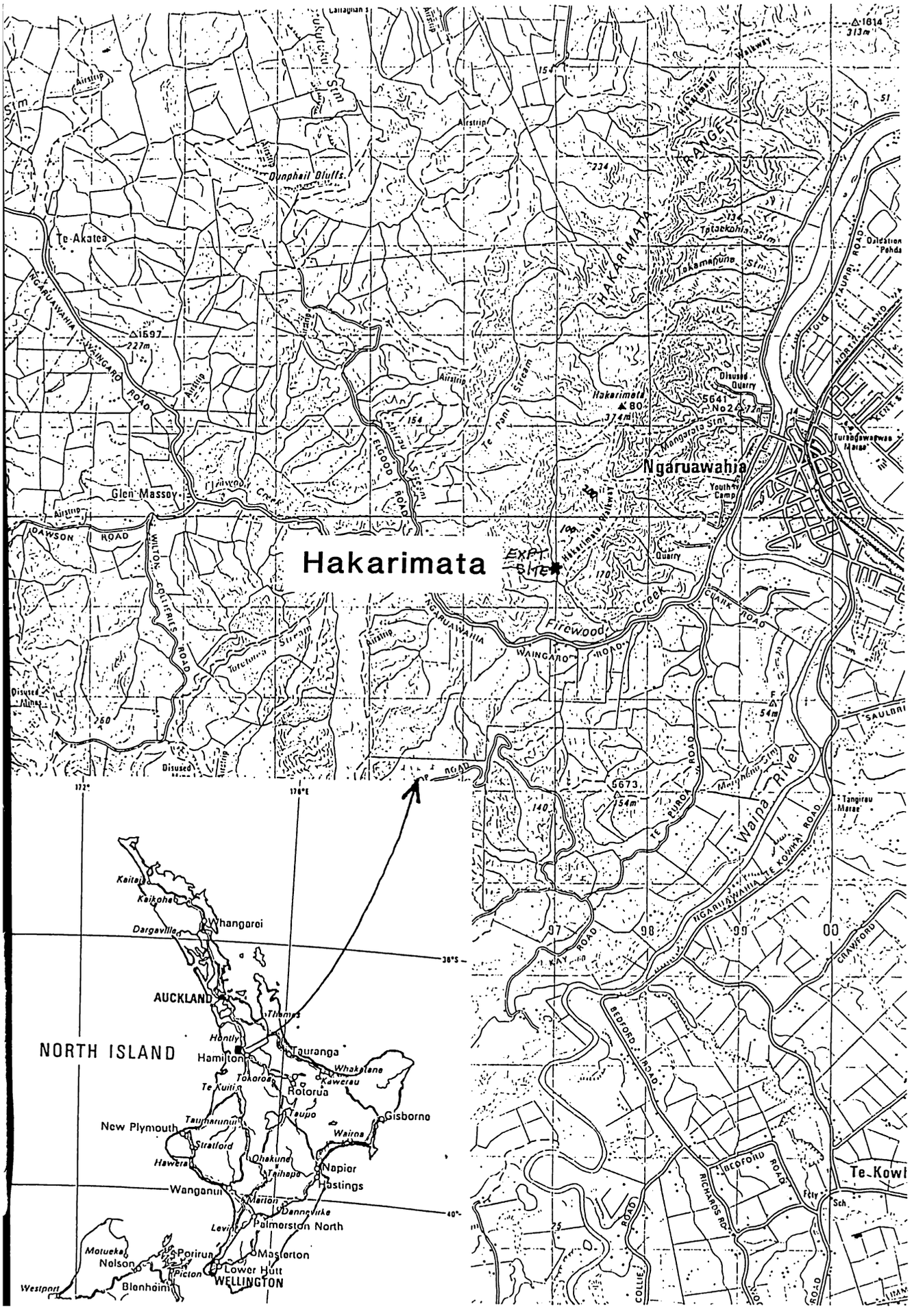
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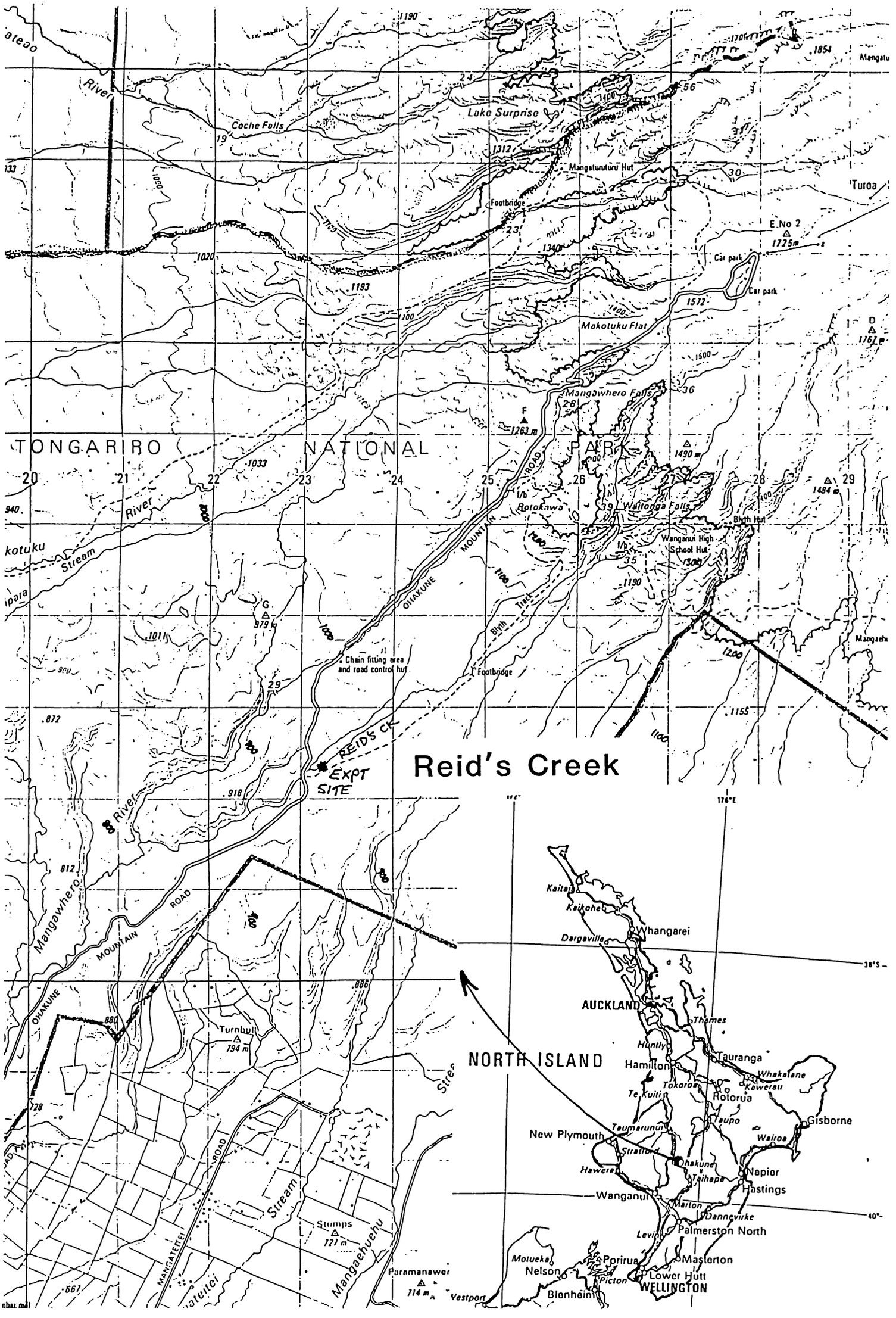
EXPT SITE
Ngaruawahia
Glen Massey
Te Akatea
Firewood Creek
Waipa River
HAKARIMATA RANGE
NORTH ISLAND
AUCKLAND
Whangarei
Hamilton
Tauranga
Rotorua
Gisborne
New Plymouth
Napier
Hastings
Wanganui
Palmerston North
Masterton
Lower Hutt
WELLINGTON
Nelson
Blenheim
Westport
Te Kowhai


Reid's Creek
REID'S CK
EXPT SITE
TONGARIRO NATIONAL PARK
Luke Surprise
Coche Falls
Mangaturuturu Hut
Footbridge
Makotuku Flat
Mangawhero Falls
Waitonga Falls
Rotokawa
Blyth Hut
Wanganui High School Hut
Chain fitting area and road control hut
Turnbull
Stumps
Paramanawe
Mangaehuehu
Stream
Makotuku Stream
Mangawhero River
Mangawhero Mountain Road
Ohakune Mountain Road
Blyth Track
Turoa
E No 2
Car park
NORTH ISLAND
Kaitaia
Kaikohe
Whangarei
Dargaville
AUCKLAND
Thames
Huntly
Hamilton
Tauranga
Whakatane
Tokoroa
Kawerau
Te Kuiti
Rotorua
Taupo
Gisborne
New Plymouth
Taumarunui
Wairoa
Stratford
Ohakune
Hawera
Taihape
Napier
Hastings
Wanganui
Marton
Dannevirke
Palmerston North
Levin
Motueka
Nelson
Porirua
Masterton
Picton
Lower Hutt
WELLINGTON
Blenheim
Westport
38°S
40°S
176°E

ECOLOGY OF AQUATIC HYPHOMYCETES IN NEW ZEALAND STREAMS.

A thesis

submitted in fulfilment

of the requirements for the Degree

of

Doctor of Philosophy

in

Biological Sciences

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University of Waikato

by

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ABSTRACT.

This work investigated the occurrence of aquatic hyphomycetes in stream ecosystems in New Zealand. It provided evidence for the potential importance of detrital cycles in such ecosystems and of the role of aquatic hyphomycetes in the decay of leaf litter. Leaves from a representative range of plant species were enclosed in mesh bags in two undisturbed streams flowing through different forest types at different altitudes, and fungal population dynamics followed during leaf decay. A method was developed, using pure cultures of appropriate aquatic hyphomycetes, which enabled the mycelial content of leaf material (m mycelium/g) attributable to individual fungal species to be quickly and easily determined from the spore production measured from leaf samples. Antibiotics were used to quantify the relative fungal and bacterial contributions to microbial activity of leaf samples through the selective inhibition of respiration. Fungi accounted for about half of the total microbial respiration, and on average 80 % of this fungal respiration was attributable to aquatic hyphomycetes.

Three major patterns were apparent from the data for aquatic hyphomycete population dynamics. 1) A successional pattern was identified among aquatic hyphomycetes, with the species falling into three groups on the basis of the order of their period of abundance. 2) Aquatic hyphomycete abundance varied among leaf types, with soft-rotting types (hangehange, young kanono) being poorly colonised compared to firmer, darker-rotting types (old kanono, kamahi, silver beech, tawa, kauri, and rimu). Nikau (palm) and ponga (fern) leaves, although not soft-rotting, were also poorly colonised by

aquatic hyphomycetes, but bore a rich alternative mycoflora. 3) The occurrence of many aquatic hyphomycetes showed seasonal preferences and a distinct geographical distribution which followed that of higher plants. Both seasonal and geographical patterns were probably related to temperature.

Laboratory evidence was obtained of the potential importance of oxygen in determining aquatic hyphomycete occurrence, with the dominant species in most New Zealand streams (*Lunulospora cymbiformis*) being extremely intolerant of low oxygen conditions.

Two new species of aquatic hyphomycetes were isolated in the course of the work.

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CHAPTER ONE: INTRODUCTION.

Aquatic hyphomycetes are a group of fungi whose primary habitat is in streams, most commonly on decaying leaves. In their widest sense they consist of fungi with distinctive branched or sigmoid conidia which were first discovered in streams or rivers, together with a few species having simple conidia, but which inhabit the same niche as most of the species with complex conidia. Thus they are a loosely defined group, not a single taxonomic division, nor entirely ecologically uniform. Most are probably active in leaf decomposition, a number having been shown to produce pectinases and cellulases (Suberkropp and Klug, 1976; Singh, 1982; Chamier, 1985), although fewer species (six out of sixteen tested by Fisher et al, 1983) have been found capable of lignin dephenolization. Although most commonly called "aquatic hyphomycetes", the group has also been known as "Ingoldian conidial fungi", "freshwater hyphomycetes", and "amphibious hyphomycetes" (Aimer, 1982; Aimer and Segedin, 1985b; Webster and Descals, 1981). Most are known only as anamorphs.

Kaushik and Hynes (1968, 1971) and Triska (1970) used antibiotics to measure the relative importance of bacteria and fungi in decaying leaves in streams, and found fungi to dominate in most cases. Other workers have indicated that aquatic hyphomycetes are probably the dominant fungal group on decaying autumn shed leaves in clean streams in the Northern Hemisphere (Bärlocher and Kendrick, 1974; Suberkropp and Klug, 1976; Ingold, 1976; Willoughby, 1978), mainly on the basis of the frequency with which they were observed. However, a number of phycomycetous fungi have also been isolated

frequently from aquatic habitats, some at least being cellulolytic and potentially capable of competing with aquatic hyphomycetes (Thompstone and Dix, 1985). A number of conidial fungi other than aquatic hyphomycetes have also been isolated frequently from submerged leaves in previous studies, for example Chamier et al (1984). One of the aims of the present work was to quantify the aquatic hyphomycete biomass present in leaves in streams, and to measure the relative importance of aquatic hyphomycetes compared with other microbial groups.

Successive workers have found aquatic hyphomycetes to be most abundant in clean, well aerated, flowing streams (for example, Nilsson, 1964). However, little or no previous work has been done on the effects of oxygen concentration or of nutrient enrichment on aquatic hyphomycetes under experimental conditions. In the present work experiments were designed to test for the effects of these factors.

Within the clean, well aerated stream category some differences in distribution of species or of species richness have been observed between stream types in previous studies. For example, Nilsson (1964) noticed differences between streams of different sizes, altitudes, and vegetation types. Wood -Eggenschwiler and Bärlocher (1983) found a correlation between species richness and stream pH. Suberkropp (1984) related some of the differences in species composition between sites in his study to differences in temperature. In the present work two streams were chosen for intensive study, and a wider range were sampled once only, to look for similar patterns in New Zealand streams to those found overseas, and to provide a data base of aquatic hyphomycete occurrence in undisturbed stream habitats for comparison in future work.

A number of overseas studies, and one from New Zealand (Aimer

and Segedin, 1985a) have shown a seasonal pattern in spore concentration in stream water. Higher spore numbers occurred in autumn and winter in all studies. This was related to substrate levels in the streams. However, a similar seasonal pattern appeared to occur in the number of spores produced per g of leaves placed in the stream in mesh bags in the New Zealand study (Aimer, 1982). Thus there appeared to be some seasonal variation in abundance unrelated to substrate levels. One of the aims of the present work was to see if this pattern could be repeated, i.e. was a real seasonal effect, whether the same pattern appeared in different aquatic hyphomycetes, and whether the same pattern occurred in different stream types, for example, streams with riparian vegetation types that show different seasonal patterns in litter production.

Several overseas studies have followed aquatic hyphomycete populations on various leaf types during decay. Most found differences in the rate of colonisation or species richness between leaf types (e.g. Bärlocher, 1980; Bengtsson, 1982; Nilsson, 1964). Some evidence of successional patterns among aquatic hyphomycetes appeared in some studies (Bärlocher and Kendrick, 1974; Bärlocher et al, 1978; Bärlocher, 1982a; and Suberkropp and Klug, 1976; 1981). However, in other studies no such successional patterns occurred (Bärlocher, 1982a; Bärlocher and Schweizer, 1983; Chamier, 1987; Chamier and Dixon, 1982; Padgett, 1976). In the present work leaf bag experiments were set up to look for substrate preferences and successional patterns on different leaf types and to compare these with the patterns seen in overseas work. This work was of interest mainly because of the apparent differences in ecology between New Zealand and northern hemisphere streams, particularly in regard to the abundance of invertebrate detritivores, the major "predators" of

aquatic hyphomycetes and among their most important competitors for leaf litter (Bärlocher, 1982b; Towns, 1976). A reduction in the importance of invertebrate detritivores could have important effects on fungal dynamics.

There has been little previous work on the fate of dead leaves in New Zealand streams, in particular on the role of aquatic hyphomycetes in their decay. In overseas studies it has been possible to link rates of weight loss to sources such as leaching, microbial activity, and invertebrate consumption (Petersen and Cummins, 1974). For most leaf types invertebrate consumption appears to depend on the prior colonisation and conditioning of the leaves by microorganisms, including aquatic hyphomycetes (reviewed by Bärlocher, 1985). The relative importance of microbial or invertebrate action on leaf weight loss varied depending on the leaf type and the stream or river type (Bärlocher, 1985; Matthews and Kowalczewski, 1969). Towns (1976) suggested that invertebrates were less important than microorganisms in litter breakdown in many New Zealand streams, except where *Zelandopsyche ingens* occurred, because, apart from this species, the leaf shredding feeder category is poorly represented among New Zealand invertebrates. Winterbourn et al (1981) hypothesised that the paucity of leaf shredding invertebrates relates to the instability of many New Zealand streams and rivers. They suggested stream ecosystems utilising primarily allochthonous detrital energy sources (wood and leaf litter) directly rarely occur except in small, low gradient streams. In addition, Davis and Winterbourn (1977) found very little colonisation of mountain beech leaves by aquatic hyphomycetes in a South Island stream, although the leaves were readily consumed by *Z. ingens*. They suggested that leaves of the Fagaceae in general might prove to be poor aquatic hyphomycete substrates.

Thus another of the aims of the present work was to examine these ideas, particularly as they applied to aquatic hyphomycetes in New Zealand. A leaf bag experiment was set up to measure decay rates for different leaf types, together with general microbial activity (respiration), and colonisation by aquatic hyphomycetes in particular, in two different streams, while collections of naturally occurring litter and water samples from a variety of streams allowed an estimation of the potential for a detrital cycle in New Zealand streams, particularly those in the North Island.

One of the major problems of fungal ecology is the measurement of biomass in natural substrates. Such measurements were required in the present work. The different possible techniques have been reviewed by Matcham et al (1984). Only three undeveloped techniques had the potential to measure different genera or species separately, namely: use of specific fluorescent antibodies, electrophoretically separable proteins, or ELISA or RIA techniques. The alternative was to use frequency of isolation data to subdivide a general fungal biomass measurement, such as mycelial lengths or respiration. Frequency of isolation data is timeconsuming to obtain, selective for species growing and competing on the medium type used, and may be influenced by levels of dormant propagules rather than active mycelium. In addition, mycelial lengths are difficult to measure in hard natural substrates such as leaves. Thus an alternative method was sought.

Aquatic hyphomycetes generally produce abundant, readily identifiable spores. For this reason studies of aquatic hyphomycetes on leaves in streams have quite commonly used the number of spores produced during a brief incubation period as a measure of relative abundance, for comparative purposes. For

example, Willoughby (1978) suggested that the maximum rate of sporulation obtainable from substrates under constant conditions could be used as a quantitative assessment of the aquatic hyphomycetes present. The technique is generally criticised for selecting for prolifically sporulating species, and for probably being influenced by the substrate type and stream factors (Chamier et al, 1984; Bärlocher, 1982a). It was decided in the present work that it might be possible to quantify and compensate for these factors, and to use spore production as a measure of vegetative biomass. Experiments were carried out to calculate biomass (mycelium)/spore conversion factors for individual species, to allow for differing sporulation capacities between species. Following this, conditions which differed between pure culture (in vitro) and naturally occurring leaves (in vivo) were tested for their effect on the mycelium/spore conversion factors derived from pure culture.

Further experiments were undertaken in order to gain an understanding of the factors controlling sporulation of aquatic hyphomycetes, in pure culture and from leaves collected from streams, in an attempt to provide a theoretical basis for the method. There has been little previous work on the sporulation of aquatic hyphomycetes, and how the induction mechanism compares with those of other fungi, many of which are triggered to sporulate by unfavourable conditions which check vegetative growth, such as nutrient depletion (Smith and Berry, 1974; Cole, 1986). Nilsson (1964) found most aquatic hyphomycetes grown on high nutrient media did not sporulate unless submerged in deionised water, but that some sporulation occurred on low nutrient media. He suggested nutrient depletion induced spore production, while cutting or damaging mycelium further stimulated it. This behaviour was similar to that found in many common soil and laboratory fungi, although such fungi

do not normally sporulate under submerged conditions. The aim of the present work was to repeat and extend Nilsson's observations, using liquid culture techniques as well as different agar types.

To conclude: Chapman (1975) quoted a need in New Zealand for detailed background information on aquatic communities to provide a basis for their management. This study provides evidence of the importance of detrital cycles in New Zealand streams, and of the occurrence of aquatic hyphomycetes in this. It attempts to quantify, for the first time in New Zealand, some of the entities and processes involved. On an international scale the study furthers understanding of aquatic hyphomycete ecology and physiology in several areas, most notably through the development of a rapid technique to measure the biomass of individual species present in naturally occurring leaf litter.

CHAPTER TWO: MATERIALS AND METHODS.

The present work includes a major leaf bag experiment, a stream survey, and a number of supplementary experiments. The aims of the supplementary experiments were:

1)to examine the factors inducing and controlling sporulation,

2)to develop and test a technique for using sporulation as a measure of biomass,

3)to test some of the factors limiting the aquatic hyphomycete niche, and

4)to measure the relative contributions to microbial respiration of bacteria, fungi, and aquatic hyphomycetes.

The aim of the leaf bag experiment and stream survey was to examine the process of leaf decay, and the occurrence and importance of aquatic hyphomycetes on different leaf types, in different streams, and in different seasons, using the sporulation technique developed, together with particle plating methods.

The materials and methods used in this work are described here. A number of techniques were common to several of these experiments, and these are given in detail first (section 2.1). The setup of the more complex leaf bag and stream survey experiments are described here (section 2.2 and 2.3), while the setup of each of the smaller, supplementary experiments is outlined separately in the corresponding results sections (Chapter 4), .

2.1 GENERAL METHODS.

2.1.1 Isolating and Culturing aquatic hyphomycetes.

Most of the pure cultures of aquatic hyphomycete species used in the present work were obtained as single spore isolates from spore solutions. Two isolation methods were used. In the first, spores were picked out individually with an entomology needle or micropipette and placed on agar plates. In the second method 10 ml of spore solution was added to 50 ml of sterile water and small amounts were pipetted onto, and spread over the isolation medium. The isolation medium used was 0.2 % malt agar containing 50 ppm each of penicillin and streptomycin, or 60 ppm of chloramphenicol. Colonies which appeared to be clean were subcultured onto 1.5 % malt agar, either proprietary (BBL), or made with 15 g "Maltexo" malt extract, 15 g agar, and 1 l deionised water. Cultures were maintained on these, although after a number of transfers on either type changing to the other malt agar type gave better growth, suggesting neither was perfect. Pieces of agar were submerged in vials of sterile water for long term storage (Boesewinkel, 1976).

The cultures examined for the purpose of taxonomic description were grown on 1.5 % malt agar. Pieces of well grown colonies were cut out and submerged in sterile deionised water in petri dishes to obtain sporulating material. These could be observed directly using a stereo microscope, and slides prepared from areas showing the most abundant sporulation.

Several agar types other than 1.5 % malt agar were used in sporulation experiments: 0.15 % malt agar, water agar (15 g agar + 1 l deionised water), glucose, starch, pectin, cellulose and leaf agar. The latter five consisted of a carbon source, agar (15 g/l), and an inorganic mineral nutrient solution known as "Ruakura

solution", which was mixed from four separate stock solutions. The composition (final concentrations) of Ruakura solution was as follows:

Stock A:	$Mg(NO_3)_2.6H_2O$	0.2966 g/l
	$Ca(NO_3)_2.4H_2O$	1.0060 g/l
	N_4HNO_3	0.5119 g/l
	KNO_3	0.1367 g/l
Stock B:	NaCl	0.0198 g/l
	KH_2PO_4	0.1602 g/l
	K_2HPO_4	0.0984 g/l
	K_2SO_4	0.3971 g/l
	Na_2SO_4	0.0359 g/l
Min. El.:	H_3BO_4	4.7855×10^{-7} g/l
	$MnCl_2.4H_2O$ (acidified*)	1.5195×10^{-5} g/l
	$ZnCl_2$ (acidified*)	4.3971×10^{-6} g/l
	$CuCl_2.2H_2O$ (acidified*)	9.0582×10^{-7} g/l
	$(NH_4)_6Mo_7O_{24}.4H_2O$	1.5597×10^{-7} g/l
	$CoCl_2.6H_2O$	3.0594×10^{-7} g/l
Fe Citrate:	$FeC_6H_5O_7.5H_2O$ (acidified*)	2.376×10^{-5} g/l

*total of 1.1224×10^{-4} ml 0.1 N HCl /l

and 2.0944×10^{-4} ml 1 N HCl /l

In addition to these defined compounds a small quantity (0.05 g/l) of yeast extract was added. This nutrient solution was used at full strength in the glucose, starch, pectin and cellulose agars, and diluted by a tenth to make up the leaf agars. Stock B was

autoclaved separately to prevent precipitation.

The carbon sources in the nutrient agars were:

D (+) -Glucose (dextrose), M&B Ltd

Starch (soluble), BDH Chems Ltd

Pectin (apple), 250 grade, BDH Chems Ltd

Cellulose powder, CC31, microgranular form for column chromatography, Whatman Ltd.

The pectin was mixed with about 2/5 of the water in a blender to avoid lumps, and required separate autoclaving from the agar if the agar was to set.

The leaf agar was made from leaves collected randomly from Hakarimata stream, air dried, ground to a powder, weighed, moistened, and gas sterilised (see 2.1.2). Two concentrations of leaf powder were used: 10 g/l and 1 g/l. Agar was also made up with 10 g/l leaf powder and deionised water (i.e. no extra nutrients added).

The solution used for liquid culture experiments was Ruakura solution (with yeast extract, as above), either full strength or diluted to 10 % strength, with either 10 or 1 g/l glucose.

Ruakura solution, diluted to 1 % strength was used as the fluid in which sterile leaves inoculated with aquatic hyphomycetes were incubated. Cooper and Thomasson (1988) recorded mean concentrations of N from small streams in mixed podocarp -hardwood vegetation on the Volcanic Plateau that were approximately a tenth of those of 1 % Ruakura.

In the case of cultures grown on agar two 1 -3 mm^3 blocks of agar with mycelium were used to inoculate each plate. A spore solution was used to inoculate leaves in the sterile leaf experiment. This was obtained by submerging pieces of colonies

growing on agar in 150 ml deionised water for 4 -5 days. Spore solutions gave very uneven numbers of colonies per flask when used as the inoculum for liquid culture, so a mycelial inoculum was used instead. A spore solution was grown in full nutrient solution for one week at 13 C and the mycelium filtered off. This was divided up by eye in a sterile petri dish into equal sized lots (with dry weights which varied by about 10 %).

2.1.2 Sterilisation of leaf material.

Gas sterilisation of leaves and leaf powder was chosen as the method results in fewer biochemical changes to the substrate than autoclaving. Both ethylene oxide and propylene oxide have been used to sterilise biological materials in the past (Howard and Frankland, 1974). Ethylene oxide was used in the present work, obtained as "Fumigas", mixed with carbon dioxide to reduce the fire risk.

Preliminary work showed that immersion of freshly fallen leaves following gas sterilisation resulted in the release of massive quantities of dark brown soluble substances, which could well inhibit fungal activity, and which, presumably, would normally be carried away in a stream. For this reason whole leaves were soaked in tap water at room temperature for about two weeks prior to sterilisation, and leaves to be ground up for leaf agar were collected from in the Hakarimata stream, where they would already be leached. Although the leaves were dried for grinding, they were remoistened for sterilisation.

Simply gassing a flask with leaf material in it, sealing, and leaving for 24 hours was found to be insufficient to sterile the material. By partially evacuating the flask (and therefore the leaf

material) several times and replacing the atmosphere with Fumigas better infiltration of the leaf material was obtained. The flask was left overnight or longer, and the gassing procedure repeated on the following day. On the third day the flask was flushed with sterile air by again partially evacuating the flask and then refilling with air filtered through a 0.22 μm Millipore filter. The leaves or leaf powder were used a day later.

2.1.3 Mycelial measurements.

Calculation of the mycelial biomass/spore conversion factors from agar cultures required the measurement of the amount of mycelium present, as well as the number of spores produced. The mycelium was measured from three replicate sets of one or more 5 mm diameter agar discs identical to those from which spore production was measured. After calculating the volume of each disc they were chopped up roughly in 10 ml beakers. A small quantity of trypan blue in water was added (about half the final volume added, that is 0.2 -0.5 ml, depending on the volume of the discs and the density of the mycelium) and the contents sonicated (setting 4) until smooth. The rest of the liquid was added and mixed in with a pasteur pipette before placing a drop on a haemocytometer. This method gave good dispersal of the hyphae, without fragmenting the hyphae to the point that they were hard to identify as hyphae.

Counting the number of hyphal intercepts of the grid lines, following the method of Olson (1950), gave a measurement of the amount of mycelium in 0.1 mm^3 of the sample, and thence of the whole sample. Initially six fields from each sample were examined, and intercepts on both horizontal and vertical grid lines counted. This was reduced by half in later work without affecting the variance substantially.

2.1.4 Measurement of spore production.

Much of the present experimental work involved the measurement of spore production from agar or from leaves.

Leaves were washed in running tap water, and either chopped up roughly into slices about 5 -10 mm in diameter, or, in earlier work, including much of the leaf bag experiment, 5 mm diameter discs were cut from them. The resulting material was mixed and divided among three replicate flasks. About 0.3 g (D.W.) of leaf material was used per flask in standard incubations (dry weight obtained at the end of the experiment).

Cultures growing on agar were usually sampled by cutting three to six 0.5 cm diameter discs from each species for each replicate flask. Normally three species were tested at a time in each flask. Except when deliberately testing "old" mycelium (from immediately about the point of inoculation) discs were cut from just in from the colony margin. In some species a 0.5 cm disc from the colony margin consisted almost entirely of colourless mycelium from the zone of extension, but in slower growing species a large part of each disc contained mature mycelium, pigmented, and with abundant aerial mycelium. In some early experiments the colonies were cut into pie slices, and a few slices incubated in each flask.

Both leaf material and agar were incubated at 13 C in 150 ml of deionised water in 250 ml flasks, sterile in the case of the agar cultures. Some specific experiments examined the effect of different incubation fluids, but deionised water was the standard. In the standard method the flasks were shaken slowly, so that the water moved, but the leaf material or agar did not. The incubation fluid was replaced daily in the standard method.

Spore concentration was measured by drawing 10 ml aliquots of spore solution through a filter (5 μm Millipore, 13 mm diam.).

Suction, using a syringe, rather than pressure was used as this gave more even dispersal of spores. Filters were mounted whole in trypan blue in lactic acid for counting. Where there were very few spores present a low power scan (x 100) of the whole filter was sometimes used, although some smaller tetraradiate spore types required higher magnifications to be identified reliably, as did poorly stained filters, and those with much non-fungal material on them. Because the distribution of spores over the filters was not always even, usually increasing towards the margins, the high power (x 400) fields counted were arranged so the number of fields increased towards the edge of the filter in proportion to the increasing fraction of the filter area in the more marginal areas.

2.1.5 Measurement of spore concentrations in stream water.

Samples of stream water were taken from the main flow of a stream and were filtered immediately. The technique used to measure spore concentrations in streams was basically the same as that for measuring it in sporulation flasks, except that the volume filtered was larger, up to 5 l, but more normally 1 to 2 l in lowland streams, necessitating a larger Millipore filter size (47 mm diameter). A modified hand operated pump was used to suck the water through the filter. Filters were stained immediately with a small quantity of trypan blue in lactic acid, and transported back to the laboratory in petri dishes. They were then cut into eight equal pie slices and mounted in pairs in lactic acid.

Depending on the number of spores present and how clean the filter was, a pattern of selected fields was counted on high power, the number of fields increasing towards the filter margins, or one or more pieces of filter were scanned in entirety. Counting about

100 -150 spores gave a standard error for the total number of spores/l of 10 % of the mean in both the Hakarimata stream and Reid's Ck. The number of species recorded in each stream continued to increase steadily even after several hundred spores were counted, although about half were recorded in the first 50 spores seen.

The method was a modification of that originally developed by Iqbal and Webster (1973).

2.1.6 Standard method for the selective inhibition of respiration by antibiotics.

Leaves were collected from Hakarimata stream or Reid's Ck, washed gently in a tray to remove silt and invertebrates, chopped into 1 -2 cm square pieces which were mixed and divided into lots of about 0.1 -0.2 g dry weight. Each sample was placed in 20 ml of 2 g/l glucose in 10 % strength mineral medium (section 3.1.2), with or without added antibiotics, in a 30 ml McCartney bottle with a rubber septum in the lid. The antibiotic treatments used were:

Control (no antibiotics),

Antibacterial (chloramphenicol),

Antifungal (cycloheximide and nystatin),

Antibacterial and antifungal (chloramphenicol, cycloheximide, and nystatin).

The normal concentrations were 60 ppm chloramphenicol and 50 ppm cycloheximide and nystatin, but on two occasions higher concentrations, 200 ppm and 250 ppm (of all three antibiotics), were used. The bottles were sealed and incubated at 13 C. Gas exchange between the water and air phase was improved by holding the bottles on a slant to increase the surface area of the water, and shaking them gently, so that the water moved but the leaf pieces did not.

At intervals during the incubation, which continued for up to 96 hours, the oxygen concentration of the gas phase was measured by removing a 0.1 ml sample by syringe and injecting it in a Carle Basic Gas Chromatograph. When the oxygen concentration dropped below 15 % the tops were removed and the bottles reoxygenated, before continuing the incubation. Fresh nutrient and antibiotic solutions were added after approximately 24 and/or 48 hours.

2.1.7 Statistics.

In most of the present work three samples were used for the measurement of sporulation from cultures and from leaves, and of the mycelial content of agar discs. A mean and standard deviation were calculated from the three measurments, to allow a rough statistical comparison between treatments (S. D. x 2.48 = 95 % confidence limits). However, this may not have been strictly correct, as there was insufficient data to determine whether spore production or mycelial content were normally distributed or not. It was clear by combining all the experiments in which ratios (mycelium/spore values) were calculated for *L. cymbiformis* and T. chaetocladium that the ratios at least were not normally distributed, but were skewed. A high level of variability was also encountered, in spore production figures particularly. Greater significance was therefore given to the same results occurring in several species or on repetition of the whole experiment than statistical significance (i.e. non -overlap of 95 % confidence limits).

The standard error in total spore production from a normal leaf sample size in the leaf bag experiment (about 0.3g) was measured as 25 % of the mean. To lower this to 10 % of the mean would have

meant using 3 -4 times as much leaf material. It is clear from the size of the standard error in relation to the mean that only quite large effects on the total spore production of leaves will be detectable as statistically significantly different. As the number of spores of individual species will be less than the total number, effects on individual species will be statistically discernible only if the species is one of the most dominant species, and the effect very pronounced indeed. Once again, then, greater weight was given, in analysing the results of the leaf bag experiment, to the same effect occurring on more than one occasion than to statistical significance.

2.2 LEAF BAG EXPERIMENT.

This experiment followed the decay of various leaf types in mesh bags placed in two streams, the Hakarimata stream and Reid's Ck, over an 18-month period, from December 1982 to May 1984. A number of stream factors were measured regularly in conjunction with those measured from the leaves themselves. The main aim of the work was to measure the variations in aquatic hyphomycete biomass during decay and to relate these to the decay process. The variation in biomass between a range of leaf types and between two different streams, in a lowland podocarp -hardwood stream and a higher altitude beech stream was also of interest.

2.2.1 Site description and experimental setup.

The Hakarimata stream was a small unnamed stream in the southern part of the Hakarimata Reserve, map reference NZMS 1

N56/630602, altitude 75 m. The Hakarimata ranges are composed of indurated stilstones and some sandstones (Dept of Lands and Survey, 1984). The vegetation is mixed hardwood -podocarp forest, virtually unmodified immediately surrounding the stream studied. A detailed species list has been published by Gudex (1962). The stream base consisted of pebbles and sand predominantly. The pH was measured at (16/2/85) 7.6, but other stream measurements suggested this may have been slightly higher than normal. In 16 monthly visits the stream discharge at the experimental site averaged 0.013 m^3/sec.

Reid's Ck was a small stream in Tongariro National Park near Ohakune, map reference NZMS 1 N121/957565, altitude 900 m. The basal rock type was Ruapehu andesite (Geological Map of N.Z. Sheet 8, D.S.I.R. 1960). The riparian vegetation was dominated by silver and mountain beech. A list of species observed upstream from the experimental site is given in Appendix 1. The stream base consisted largely of cobbles and gravel. The pH was measured at 7.5, although Michaelis (1983) recorded 6.9 in the nearby Mangawhero river, which Reid's Ck flows into. In 16 monthly visits the stream discharge at the experimental site averaged 0.036 m^3/sec.

The slightly faster discharge rate compared to that of the Hakarimata stream reflected the higher periodic fluxes occurring in Reid's Ck. Less litter accumulated in Reid's Ck compared to Hakarimata, possibly partly due to the difference in discharge, but also to the difference in vegetation: ponga and nikau fronds, present in Hakarimata but absent from Reid's Ck, frequently formed detrital dams which accumulated smaller leaf litter. The canopy was closed over the Hakarimata stream throughout its length, while that over Reid's Ck was more sparse, with markedly fewer trees directly overhanging the stream.

Sites were chosen in each stream on the basis of the depth and

width required to accommodate the experimental setup in one place. These were shaded "pool" sites, but with appreciable flow through the parts containing the experimental setup. Some silting occurred beneath the boxes of the setup in both streams, and litter acccumulations had to be regularly cleared from the Hakarimata site. More importantly however, the boxes remained submerged in flowing water all year in both streams.

The attachment of the leaf samples had to be arranged so as to withstand periodic floods, but to otherwise mimic naturally occurring accumulations of litter in the stream, as far as access to microbial and invertebrate populations, oxygen levels, temperature, etc were concerned. The attachment was also designed to keep the presentation in the stream relatively constant throughout the 18 month experiment, and to be easily collected. Metal posts (Waratahs) were hammered into the stream bed or bank and two strands of number 8 fencing wire strung between them, so that galvanised chicken mesh boxes (20x20x7cm) could be attached at top and bottom by "Hurricane" fence clips. The 2 mm plastic mesh (shade cloth) bags enclosed in the boxes were held edge on to the current by the boxes, but for additional security were themselves attached to the fencing wire by coarse nylon fishing line. Each leaf sample (of the major leaf types) was divided among three mesh bags (20x20cm) to avoid having a large compacted mass of leaves which could become anoxic in the centre. There were six or nine bags in each box. Depending on the number of leaf types being sampled each time, one or two boxes were removed at each sampling period, and taken back to the laboratory in plastic bags to prevent dehydration. Plastic ear tags threaded on the nylon fishing line identified each box for collection. The leaf types were identified by labelling on the plastic clips closing the bag, and by colour coded plastic paperclips placed in the bags.

The leaf types used were:

Fresh kamahi (*Weinmannia racemosa* Linn. f.)

Senescent kamahi

Fresh beech (*Nothofagus menziesii* (Hook. f.) Oerst.

Senescent beech

Fresh hanghange (*Geniostoma ligustrifolium* A. Cunn.

Fresh, young kanono (*Coprosma australis* (A. Rich.) Robinson

Fresh, old kanono

Fresh, young kauri (*Agathis australis* Salisb.)

Fresh, old kauri

Fresh tawa (*Beilschmedia tawa* (A. Cunn.) Benth. et Hook. f. ex Kirk

Fresh rimu (*Dacrydium cupressinum* Lamb.)

Fresh nikau (*Rhopalostylis sapida* Wendl. et Drude)

Fresh ponga (*Cyathea dealbata* (Forst. f.) Swartz

All leaves were picked from trees close to Hakarimata or Reid's Ck (beech leaves), except for the kauri leaves, which were obtained from a tree in the Waitakere Ranges (Auckland). The leaves were stored in plastic bags in a refrigerator for a week or less before placing in the streams. The fresh kamahi and beech leaves comprised all attached leaves except obviously soft new leaves. The fresh kamahi leaves collected for the second run (leaves immersed in May 1983) were perhaps smaller on average than those picked for the first run (leaves immersed in December 1982), were more chewed (insect damage), and had less sooty mould. The individual bags contained 3 -4 g (dry weight) of kamahi or nearly 1 g (dry weight) of beech leaves. The senescent leaves were collected from the ground under trees and included only those leaves that were freshly

fallen, still yellow or partly yellow. Only one mesh bag of senescent leaves was used per collection, each containing about 7 g kamahi or 1.5 g beech. Only year old (at least) leaves were collected for the hangehange and old kanono samples, bags of each containing about 1 g and 4 g respectively. The young kanono leaves varied from soft, pale, opening leaves, to firm, but very bright green leaves, all of the current years growth (2.5 g per bag). The old kauri leaves were those four or more years old, while the young leaves consisted of the current years mature growth only. Bags contained the same number of leaves of each, weighing about 1.5 g (young leaves) and 3.5 g (older leaves). The tawa leaves were collected as for fresh kamahi, and bags initially contained about 3.5 g of leaves. Although there were no sooty moulds present, unlike the kamahi leaves, many tawa leaves supported green epiphytes (lichens etc). Individual leaves were not used in the cases of rimu, where they were too small, and nikau and ponga, where they were too large to use the leaf bag method of presentation. A small lower branch of rimu was chopped into 5 -10 cm lengths, leaving out the older, woody parts, and about 5 g of these branchlets were used per bag for rimu. The oldest living frond of nikau and ponga on a plant of each were selected and 10 cm lengths cut from it, leaving out the stipe and rachis. In the case of nikau this frond was blotched brown, and had abundant green epiphytes (lichens etc). About 4 g of nikau lamina were used per bag, and about 3.5 g of ponga per bag.

Sufficient samples were put in the stream for twelve months of collections during two overlapping periods, beginning in December 1982 (the first run), and the following May (the second run). The fresh kamahi and beech were collected after one and two weeks immersion from Hakarimata, and subsequently at monthly intervals

from both streams. Senescent beech leaves were placed in Reid's Ck only, while senescent kamahi and the remaining leaf types were placed only in Hakarimata. The senescent leaf types were used in the second run only, and were collected after one and two weeks, then 1, 2, 4, 8, and 12 months from Hakarimata, and after 1, 2, 4, 7, and 11 months from Reid's Ck. The other leaf types were used only in Hakarimata. Hangehange, kanono, and kauri were used in the first run only, and collected after one and two weeks, then 1, 2, 4, 8, 12, and 18 months, or until no leaf material remained. Tawa, rimu, nikau, and ponga leaf types were used only in the second run, and were collected after 5 and 12 months.

2.2.2 Stream measurements.

In conjunction with the monthly collection of one or more boxes of leaf samples a number of in-stream measurements were made. A maximum/minimum thermometer recorded the highest and lowest temperature over the previous month, as well as the current temperature. The spore concentration of the stream water was measured by filtering, as in the standard method (2.1.5). The stream discharge rate was measured roughly by measuring the cross sectional area (depth), and the surface flow rate, of a standard length of the stream. The naturally occurring fungal flora was sampled by collecting free litter from the stream close to the experimental site. Litter occurring in pool situations was preferred, but not that buried in silt or gravel. This litter was returned to the laboratory and spore production from it measured according to the standard method.

2.2.3 Measurements made from the leaf collections.

Four factors were measured from the collected leaf material: weight loss, microbial respiration, invertebrate numbers, and spore production. In addition, notes were kept on the visible signs of leaf decay.

The initial weight of the contents of one of the mesh bags of each sample was recorded prior to immersion in the stream. On collection, the leaves from this bag (marked by colour coded paper clips to identify the leaf type) were washed carefully to remove sand and silt, dried at 80 C for three days, cooled in a dessicator, and weighed.

In the leaf bag experiment microbial respiration was measured in BOD bottles to allow the measurement of relatively large quantities of whole leaf material, compared to the technique used in the selective inhibition of respiration experiments, in which leaves were chopped up and mixed to reduce the variation between small samples. Prior to measurement the leaves were washed gently to remove silt and invertebrates, but left entire to reduce the amount of disturbance to the microbial population prior to measuring respiration. They were placed in BOD bottles filled with stream water, and a plastic mesh sleeve added to keep the leaves from being macerated by the stirrer on the oxygen probe. Extra bottles of stream water without leaves acted as controls. There was insufficient material for replication. The bottles were allowed to equilibrate unsealed at 13 C for about an hour, then the oxygen concentration was measured using an oxygen probe, the bottles were sealed, incubated for several hours, and the oxygen measurement repeated. The length of incubation depended on the amount of material present, so that at the beginning of the experiment this was short (3 -4 hours), but lengthened to 20 hours towards the end.

A sufficient length of time was required to give a measureable difference in oxygen concentration, but not so great that the oxygen concentration dropped below about 6 ppm, in case the low levels of oxygen inhibited respiration.

When the leaves from the preweighed bag of each sample were washed this was done in a shallow tray filled with water. In this way the number of invertebrates present in each bag of leaves could be counted at the same time.

Spore production by the fungi present was measured by the standard method.

2.2.4 Particle plating to measure frequency of occurrence of aquatic hyphomycetes.

On two occasions during the leaf bag experiment extra bags of kamahi and beech were collected to allow the use of plating techniques to measure the occurrence of aquatic hyphomycetes and other fungal groups. These were July and December (1983). Some preliminary work was done using material from the February (1983) sample to determine the length of time required for surface sterilisation of different leaf types.

Discs (0.5 cm diameter) were cut from all leaf types still approximately entire at the time of sampling, otherwise the remaining veins were cut into pieces for use. All were washed thoroughly, to remove as many spores adhering to the leaf as possible, before plating. In addition, where the leaves were relatively entire further discs of each leaf type were surface sterilised prior to plating. The methods used followed Hettige (1981).

To wash the discs, 100 discs were placed in 100 ml sterile

water and shaken gently for an hour at 13 C. Following this they were washed in running (tap) water for half an hour, then rinsed in sterile water, blotted dry on sterile filter paper, and plated out, five discs per plate, on 0.15 % malt agar with 50 ppm penicillin and streptomycin. It was intended to plate the leaf washings (the original 100 ml of sterile water) as well, but this was not very important to the experiment and was omitted for lack of time.

To surface sterilise discs they were swirled briefly in a flask of 70 % alcohol and the alcohol poured out immediately. Sodium hypochlorite (1 %) was added, and left for 0, 15, 30, 45, or 60 seconds, depending on the condition of the leaves. Following this the discs were washed ~~to~~ in two lots of sterile water, blotted dry, and plated out as for the washed discs.

In the July plating, discs of kamahi and beech from Hakarimata (second run) were left for 30 seconds in the hypochlorite treatment, while those from Reid's Ck (second run) were left for 60 seconds, and the first run beech was dipped in alcohol only and the hypochlorite treatment omitted. In the December plating only discs from the second run beech from Reid's Ck were surface sterilised, for 45 seconds in the hypochlorite treatment. Only discs of second run kamahi leaves from Hakarimata were surface sterilised, for 15 seconds. However, in-order to test the effect of the one day delay normally occurring between the collection of the leaves and their plating out, a subsample of the leaves were surface sterilised and plated out on the day of collection, so that there were two sets of surface sterilised leaf discs.

A visual assessment of all the plates one week after plating was made to determine the frequency of isolation of different fast growing colony types. In the December plating a selection of these was subcultured, and later submerged in sterile stream water along

with pieces of autoclaved grass, to stimulate sporulation by phycomycetous fungi, for identification purposes.

About four weeks after plating, pieces of agar from under and around the leaf discs were cut out and submerged in petri dishes of sterile deionised water. These were examined, using a stereo microscope, after about 3 and 7 days incubation at 13 C, to identify the conidial fungi present. In addition, for some leaf types, one or two slides were made from each petri dish in order to identify the smaller spored species. In the July plating the first run kamahi, first run beech (washed and surface sterilised), second run kamahi (washed only), and second run beech (washed only) from Reid's Ck, and first run kamahi, second run kamahi (washed and surface sterilised), and second run beech (washed only) from Hakarimata were all submerged, but slides were made only for washed second run kamahi from Hakarimata. In the December plating agar pieces from all leaf types plated out were submerged and had slides made from them.

2.3 STREAM SURVEY.

The aim of this section of the work was to test for patterns of aquatic hyphomycete species distribution among undisturbed stream types and relate these to environmental factors. The survey was carried out over three years, during the summer (December to March). Twenty eight streams were sampled from throughout New Zealand (southern Westland to Auckland), largely in national parks and reserves. Nine factors were used to describe the streams: location (latitude and altitude), riparian vegetation type, temperature, pH, the biological oxygen demand of the water, colour and clarity of the

water, sediment type, and discharge or stream size. The aquatic hyphomycetes present were assessed by filtering a water sample from the stream, as in the standard method for measuring spore concentration. A litter sample was collected from most of the streams and incubated to measure spore production according to the standard method.

CHAPTER 3: Results and discussion 1- Taxonomy.

Some 36 species of aquatic hyphomycetes were recorded in the present study. A list of these follows:

Alatospora acuminata Ingold

A. ?pulchella Marvanová

Anguillospora crassa Ingold

A. filiformis Greathead

A. longissima (Sacc. & Syd.) Ingold

Articulospora tetracladia Ingold

Campylospora filicladia Nawawi

Casaresia sphagnorum Fragoso

Clavariopsis aquatica de Wildeman

Clavatospora longibrachiata (Ingold) S. Nilsson ex Marvanová & S. Nilsson

Culicidospora aquatica Petersen

Dimorphospora foliicola Tubaki

Filosporella sp. ined.

Flagellospora penicillioides Ingold

Fontanospora eccentrica (Petersen) Dyko

Gyoerffyella entomobryoides (Boerema & Arx) Marvanová

Heliscella stellatacula (Kirk ex Marvanová & S. Nilsson) Marvanová

Heliscus lugdunensis Saccardo & Therry

Lemonniera aquatica de Wildeman

Lunulospora curvula Ingold

L. cymbiformis Miura

L. ovalis sp. ined.

Margaritispora aquatica Ingold

Mycocentrospora acerina (Hartig) Deighton

M. clavata Iqbal

Taeniospora gracilis Marvanová

Tetrachaetum elegans Ingold

Tetracladium marchalianum de Wildeman

T. setigerum (Grove) Ingold

Tricellula aquatica Webster

Tricladium chaetocladium Ingold

T. splendens Ingold

Triscelophorus acuminata Nawawi

Tripospermum myrti (Lind) Hughes

Tumularia aquatica (Ingold) Descals & Marvanová

Varicosporium elodeae Kegel

A number of fungi were seen in the course of the leaf bag and other experiments that could not be identified fully. These are illustrated and described here. In addition, some of the identified species had not been previously described from New Zealand, or only as detached spores (Aimer, 1985b; Tubaki, 1965; Davis and Winterbourn, 1977). These are also illustrated and described here. Many aquatic hyphomycetes have not been frequently isolated or described so that this work is important in establishing the range of variation of the species.

Alatospora species.

Fig. 3.1, 3.2, 3.3A -G.

Spore types belonging to this genus were recorded in the leaf bag and stream survey experiments numerous times. On one occasion a single spore was seen which was possibly referable to *A. flagellata* (Gonczol) Marvanová. The other species seen were *A. acuminata* Ingold and *A. ?pulchella* Marvanová. Both were seen attached in the plating experiment and two pure cultures of *A. ?pulchella* were obtained, from which the following description is derived.

Alatospora ?pulchella Marvanová (Isolate 39 from randomly collected leaves from Hakarimata str.)

Fig. 3.1A -L.

Colony growth rate 0.42 mm/day at 13C on 1.5% MA, very flat, creamy white, moist looking, no aerial mycelium, has ribbed appearance on older parts, hyaline growing margin very narrow, reverse cream, darkening towards tan on older parts, submerged hyphae hyaline, septate, branching irregularly, rather densly packed, c. 1 μm wide. *Conidiophores* hyaline, septate, 70 -250 x 1 -2 μm to first branch, rarely branched, terminating in 1 -3 phialides, and sometimes up to 2 phialides deriving from 1 -3 cells immediately below the apex, micronematous. *Conidiogenous cells* phialidic, usually apical, 1 -3, hyaline, 7.5 -19 x 1.75 -2.5 μm, usually slightly inflated or ampulliform, collarette and periclinal thickenings present, collarette 0.5 -1 μm long. The finger -like conidium initial elongates until about the length of the mature lower axis, before budding off two lateral projections simultaneously from just below the apex. These elongate and swell at the same time as the

apex elongates to form the upper axis, bending away from the two lateral arms. Septa may form before the spore is released. *Mature detached conidia* hyaline, walls thin and smooth, tetraradiate, consisting of a roughly cylindrical axis, curved or bent at or just above the point of insertion of two similar lateral arms, which are constricted at the base and radiate away from each other and the curve of the main axis. Both arms and upper axis taper gradually, the lower axis often more cylindrical, and all are 0 -2 septate. There is almost always a septum just above the lateral arm origin, dividing the upper from the lower axis. Axis 18 -42 (the lower part 10 -17) x 1.5 -2.5 μm, arms 9 -23 x 2 -2.5 μm.

Isolate 36, from the same material as isolate 39, displayed identical cultural characteristics, but differed slightly in some features of the mature detached conidia. The conidia were very slightly larger on average: axis 31 -52 x 1.5 -2.5 μm, arms 15.5 -31 x 1.5 -2.5 μm, and the axis was frequently slightly constricted immediately above the origin of the lateral arms. It seems likely that the two isolates belong to the same species.

Marvanová and Descals (1985) distinguish *A. constricta* from *A. pulchella* mainly on the basis of conidium size (typically about 50-60 μm compared with about 30 μm respectively) and shape of the upper axis (cylindrical compared with cymbiform. Thus the size of isolate 39 corresponds with that of *A. pulchella* but the conidium shape seems to lie between that of *A. constricta* and *A. pulchella*. Isolate 36 falls somewhere between the two species with regard to size, and similarly for axis shape: although not cylindrical at the point of arm origin the upper axis could rarely be described as cymbiform. Marvanová and Descals (1985) reported axis lengths down to 20 μm for aerial conidia of

A. constricta but these were also inflated to resemble *A. pulchella*. The conidia described from isolates 39 and 36 above were produced in submerged conditions. Thus the present isolates are interpreted as *A. pulchella*, but their identity is by no means unequivocal.

Of the conidia seen in the course of the leaf bag experiment and stream survey, some clearly belong to the same species as isolates 39 and 36 e.g. Fig. 3.2F (short conidiophores - 6 μm - seen a few times in isolate 36 also), and H. Fig. 3.2E, G, and J have constricted arm bases and bent axes, but also some constriction of the axes just above arm insertion, i.e. clearly *A. pulchella* -like in shape. Fig. 3.2I and Fig. 3.3D are very similar spores but lack the obvious constricted arm bases. They are thus more like *A. acuminata sensu lato* (Marvanová and Descals, 1985). It is more realistic, however, to record all these spore types, when they occur as detached spores, simply as *Alatospora spp.* *A. acuminata sensu stricto* was fairly easily distinguished in the leaf bag experiment by the fine unconstricted arms and even dark staining of the spores compared with the other spore types in which the stain usually appeared blotchy (Fig. 3.2K, L, M, N, Fig. 3.3A, B).

A number of much smaller spores (axis less than 20 x 1.75 μm) were seen in a sample from an unnamed stream in south Westland. The shape of Fig. 3.3E and G is reminiscent of *A. pulchella* but they are smaller than Marvanová and Descals (1985) record. Fig. 3.3F and C are probably deformed *A. acuminata*, as Marvanová and Descals depicted a similarly deformed, though larger, spore from pure culture (compare with Fig. 3.3B).

Fig. 3.1

Scale 1 = 50 μm.

Attached and detached conidia of *A. ?pulchella* from culture (A -L isolate 39; M,N isolate 36).

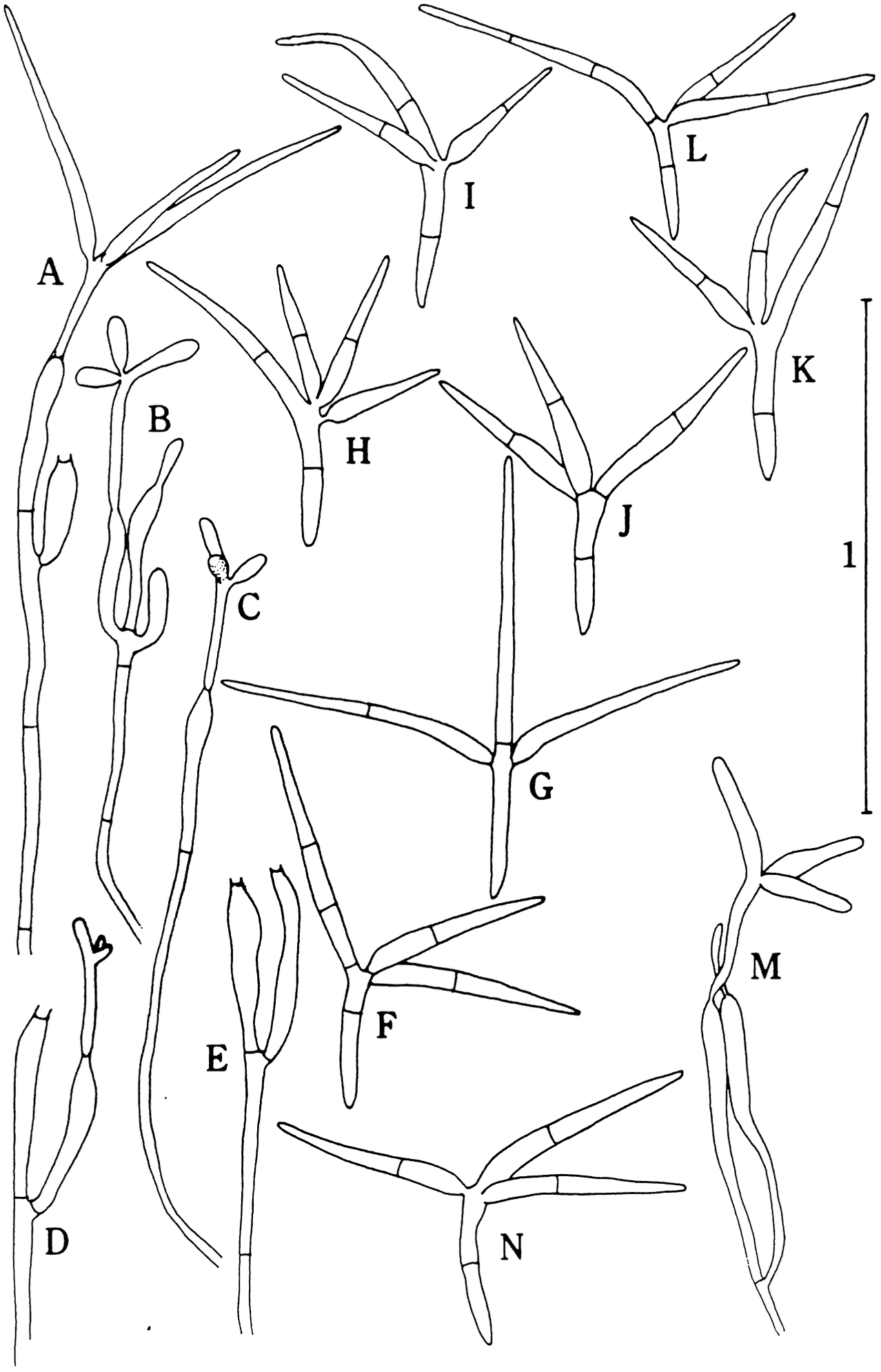

A
B
C
D
E
F
G
H
I
J
K
L
M
N
1

Fig. 3.2

Scale 1 = 50 μm.

A -D *A. ?pulchella>fo=1> (isolate 36) continued.*

E -N Spores of Alatospora produced from leaf bag leaves, all from Reid's Ck except F (Hakarimata stream) and N (Rock Burn, South Island).

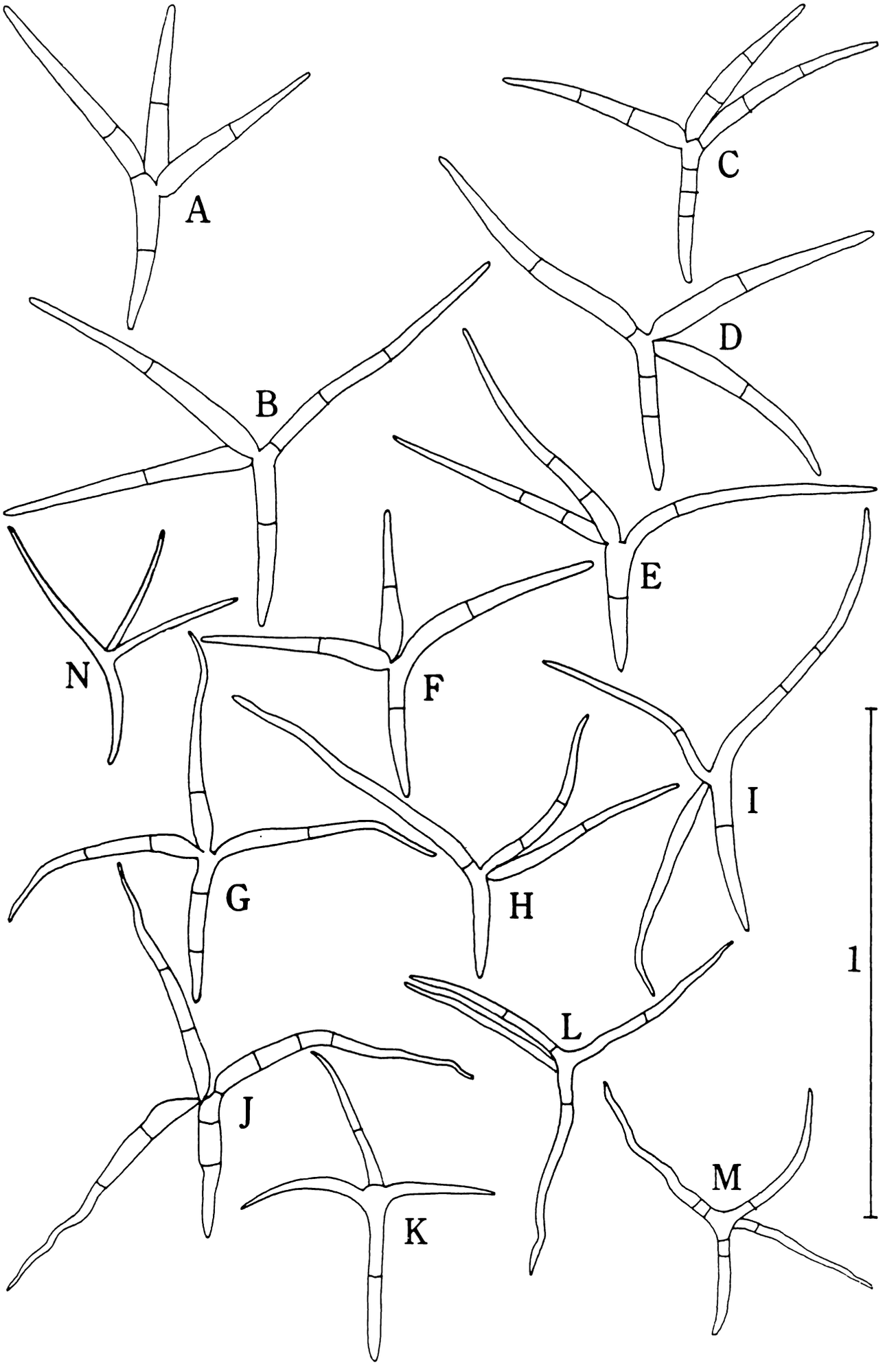

A
B
C
D
E
F
G
H
I
J
K
L
M
N
1

Fig. 3.3

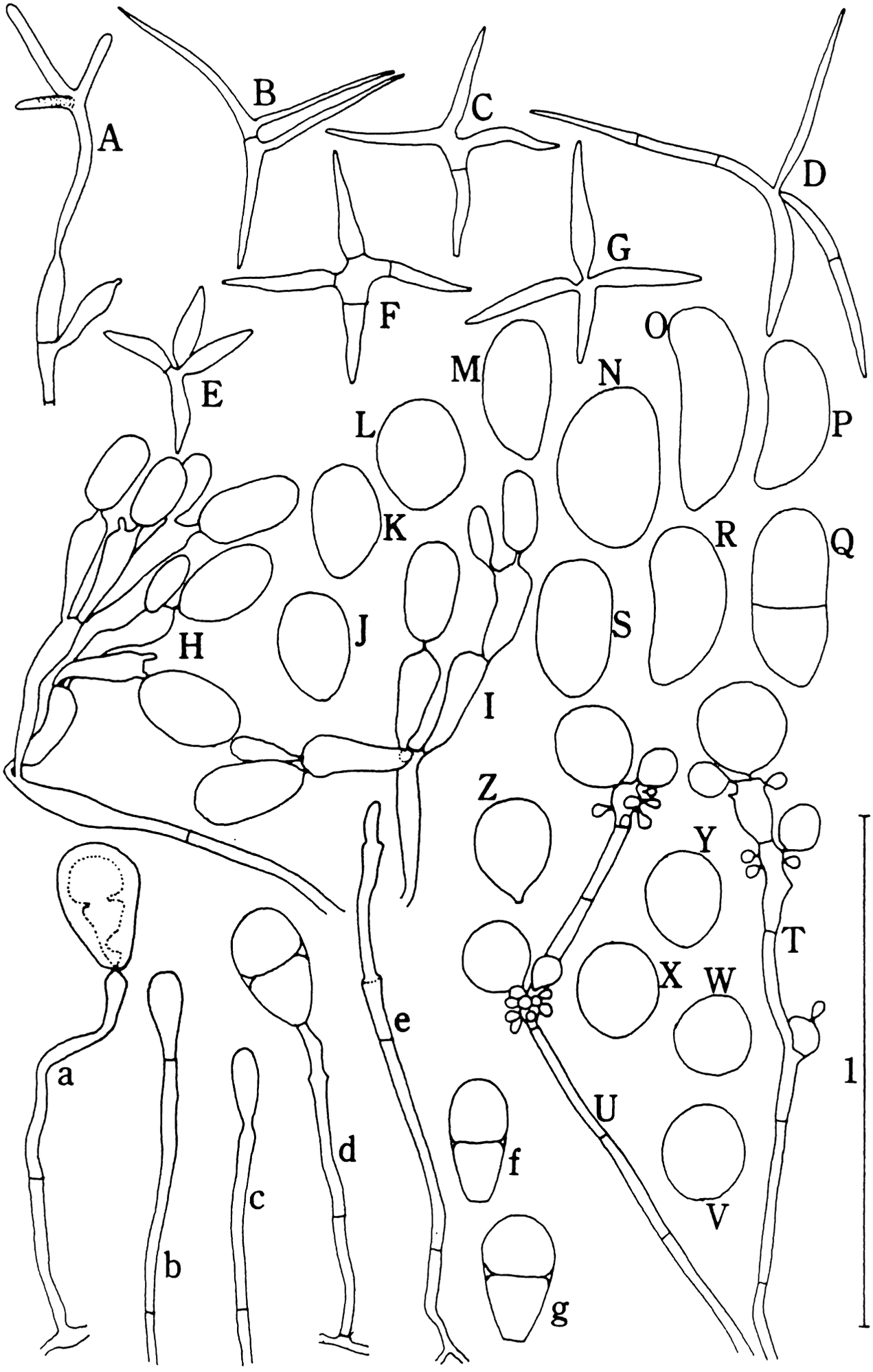
Scale 1 = 50 μm.

A -G *Alatospora* continued; A and D from beech leaves from Reid's Ck, the rest from Rock Burn (South Island) leaf (B) and stream (C,E,F,G) samples.

H -S Attached and detached conidia of *Dimorphospora foliicola* from isolation agar on which kamahi leaves from Hakarimata stream were plated.

T -Z Attached and detached conidia of Unidentified sp. 2 from isolation agar on which beech leaves from Reid's Ck were plated.

a -g Attached and detached conidia of *Endophragmia microaquatica* from isolation agar on which kamahi leaves from Hakarimata stream were plated.


A
B
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D
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K
L
M
N
O
P
Q
R
S
T
U
V
W
X
Y
Z
a
b
c
d
e
f
g
1

Fig. 3.4

Scale 1 = 50 μm.

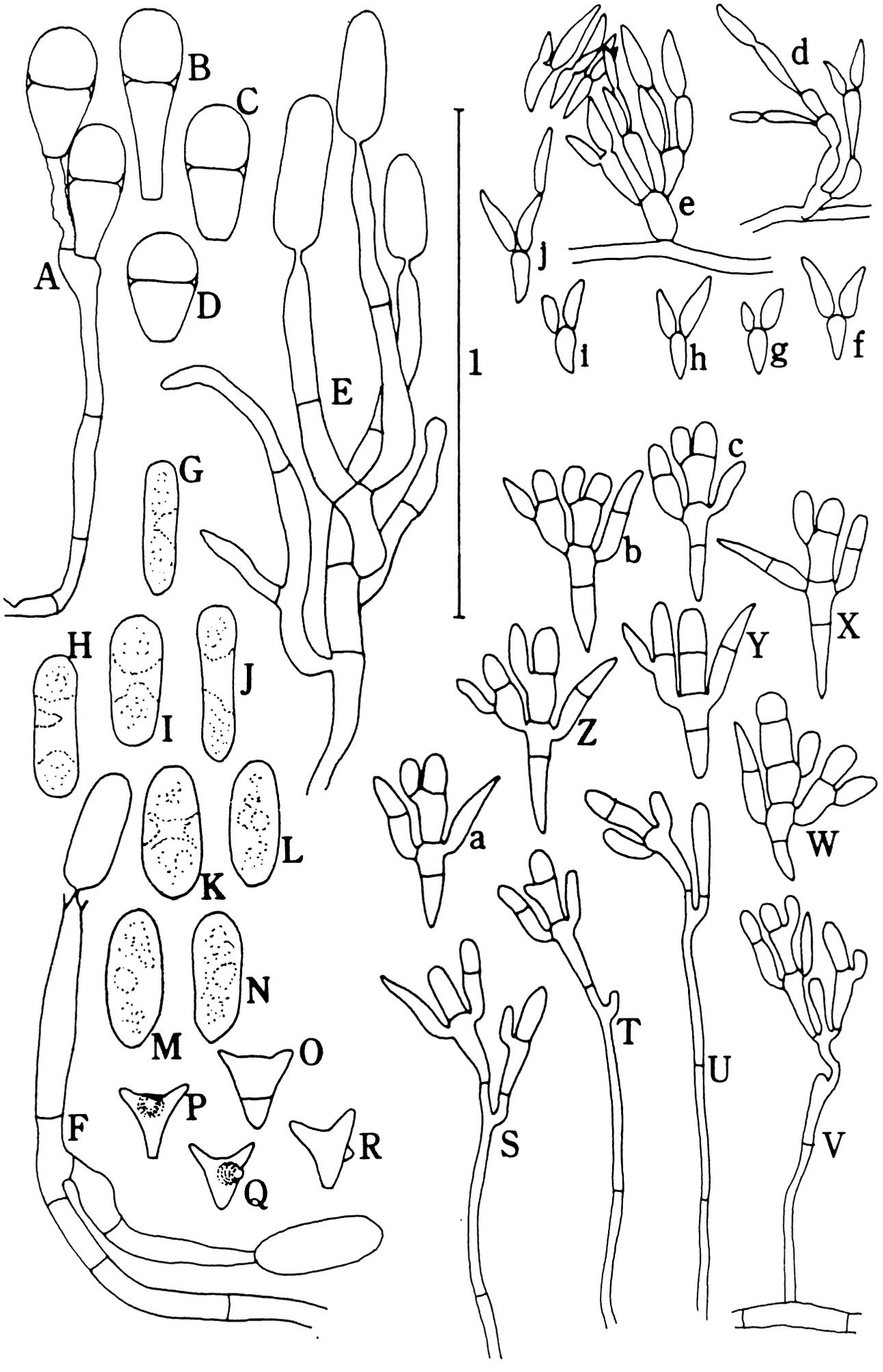
A -D *E. microaquatica* continued.

E -N Attached and detached conidia of Unidentified sp. 1 from isolation agar on which kamahi leaves from Hakarimata stream were plated.

O -R Spores of *Heliscella ?stellatacula* from beech leaves from Reid's Ck.

S -Z + a -c Attached and detached conidia of Tetracladium sp. from culture.

d -j Attached and detached conidia of *Tricellula aquatica* from culture.


A
B
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I
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K
L
M
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O
P
Q
R
F
1
a
b
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d
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f
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h
i
j
X
Y
Z
S
T
U
V
W

Culicidospora aquatica Petersen.

Fig. 3.5G, H, I.

These spores were uncommon, seen only in Reids Ck, as detached spores. The spores consist of a main axis, 3 to 4 septate, 42 -45 x 5.5 -11.5 μm wide at the widest point (apical or more frequently subapical cell) tapering to 2.5 -4 μm at attachment point. The axis bends through up to 45° at about 10 μm below the apex and a long apical process, 55 -80 x 1.25 μm issues at a further 45° angle in the same direction. Two processes, 53 -80 x 1.25 μm, arising from the same level on the subapical cell radiate, one upwards and one retrorsely. An incipient germ tube protrudes through the basal scar as another long process, 31 -45 μm long and tapering rapidly to 1.25 μm wide. All processes are broadly inserted, normally aseptate, and tapering only at the very tip. Axial cells of presumably older spores often are somewhat swollen.

According to Descals and Webster (1982b) the main difference between the two species of *Culicidospora* (*C. aquatica* and *C. gravida* Petersen) is in the method of conidium release from the conidiophore, but the difference in process length (apical and lateral processes 47 -57 x 0.5 μm in *C. aquatica* compared with 20 -27 x 2 -2.5 μm in *C. gravida* together with their general appearance indicate clearly that the present spores belong to *C. aquatica*. There are some small differences: the apical and lateral processes are recorded as having constricted bases in both species, the processes are wider in the present spores than recorded for *C. aquatica*, and the spores display a greater range of width, despite the small number seen.

The species has been recorded in New Zealand previously by Tubaki (1965).

Dimorphospora foliicola Tubaki.

Fig. 3.3H -S.

Aimer (1985b) described as "*D.* aff. *foliicola*" an unidentified fungus with a strong superficial resemblance to *D. foliicola*. In the plating experiment both *D.* aff. *foliicola* and *D. foliicola* were seen and could be compared. The following incomplete description of *D. foliicola* is derived from slides of mixed cultures from the plating experiment.

Conidiophores hyaline, septate, erect, irregularly branched or simply penicillate; *Conidiogenous cells* 1 -3, hyaline, holoblastic, apical or occasionally subapical, integrated, fusiform or more commonly clavate, 9 -16.5 x 2.5 -4 μm, bearing 1 -2 conidia developing in succession. Each conidial primordium commences as a finger -like projection which increases in length and width and is cut off by a basal septum. Secession is schizolytic and may leave a very small projection on the conidiogenous cell; *Mature detached conidia* hyaline, walls thin and smooth, broadly ellipsoidal to oval or subreniform or frequently somewhat obovoid, 10.5 -20 x 6.5 -10 μm.

This species corresponds well with published descriptions of *D. foliicola* and is thus a new record for New Zealand. It is also clearly distinct from *D.* aff. *foliicola*. The two species, as seen in the present work, differ in several significant respects: in the presence or absence of subreniform conidia and also in the presence or absence of separating cells, i.e. schizolytic or rhexolytic secession of conidia. Published descriptions of *D. foliicola* state that it is creamy on agar

whereas *D.* aff. *foliicola* is dark brownish -grey. These differences are of such magnitude that the two species would appear to differ at the generic level. Thus "*D.* aff. *foliicola*" has been placed in *Lunulospora* in the present work (see *Lunulospora ovalis* sp. ined.).

Whatever the taxonomic distance between *D. foliicola* and *D.* aff. *foliicola*, the two species can be very difficult to distinguish between in ecological experiments from detached spores unless subreniform conidia are conspicuous. Two other fungi were seen in the plating experiment which could also be confused with these fungi when seen only as detached spores. Unidentified sp. 1 was usually distinguishable by its rather granular appearance when stained compared with the uniform stain of *D.* aff. *foliicola* and *D. foliicola*. Unidentified sp. 2 is generally more spherical and slightly smaller than either, but the difference is small and the stained appearance very similar. However sp. 2 appeared to be relatively rare, so that detached spores could be identified as "*Dimorphospora* -like spp." (*D. foliicola* and/or *L. ovalis*), or as *L. ovalis* where spores were abundant but did not include subreniform types, with a reasonable margin of error.

Endophragmia microaquatica (Tubaki) Matsushima

Fig. 3.3a -g and Fig. 3.4A -D.

This species was seen regularly in small numbers in the Hakarimata stream in the leaf bag experiment and in the plating experiment, from which the following incomplete description is taken.

Vegetative hyphae hyaline to pale brown, branched, septate. *Conidiophores* hyaline to pale brown, erect, simple or rarely branched, terminal or lateral, 12.5 -75 x 1 -2 μm, 0 -few septate, micronematous. *Conidiogenous cells* holoblastic, apical, single, pale brown, integrated, not distinguishable from conidiophores, proliferation percurrent, occasionally sympodial, site of earlier conidia indicated by swelling or ridge, and possibly a septum as the stain density sometimes changes abruptly at the site, up to two proliferations seen, distance between successive conidia 2 -14 μm. The conidium initial begins as a finger -like outgrowth of the conidiogenous cell, which becomes cut off by a septum, swells, becoming clavate and finally septate, before rounding off of the basal septum releases the conidium. *Mature detached conidia* pale brown, walls thick and smooth, obovoid to clavate, attachment point flattened and prominent, 1 -septate, 10.5 -18.5 x 5.5 -8 μm, tapering to 2 -4 μm at attachment point.

Mature conidia do not take up cytoplasmic stains readily. Fig 3.3a shows an attached conidium, already thick -walled, but still taking up some stain which delimits the septum in the process of formation, growing in from the edges.

Endophragmia is the most likely genus which might accommodate the present species. It includes species with terminal, solitary, thick walled, dematiaceous, 1 -septate, turbinate conidia produced on percurrently proliferating conidiophores, for example *E. pinicola* Ellis, which differs from the present fungus in having longer conidiophores and the possession of the occasional large frills about the conidiophore, typical of most *Endophragmia* species, and formed from the ruptured base of a conidium. *E. pinicola* was described from pine needles, in

terrestrial litter. However, five *Endophragmia* species have been recorded previously from freshwater (Shearer, Crane, and Miller, 1976). *E. microaquatica* appears to be the most closely similar. Petersen's (1962) conidial widths are much wider than those of the present fungus, however those of Matsushima (1975) match the present conidia. Proliferation was relatively rarely seen in the present material compared with Matsushima's. Possibly his material was simply older. There seems little doubt that the present fungus belongs in *E. microaquatica*.

Filosporella sp.

Fig. 3.5L -W.

This fungus was seen fairly frequently in both leaf bag (sporulation) and plating experiments, although never very abundant. A pure culture was obtained from the Hakarimata stream, on which the following description is based.

Colony growth rate 0.95 mm/day at 13 C on 1.5 % MA; dark brown, very slightly olivaceous, with abundant brownish -grey aerial mycelium, growing margin hyaline and a few mm wide; reverse shades from cream margins to dark brown to a paler olive colour under the oldest parts; buried hyphae 2 -2.5 μm diam., hyaline to mid brown. *Conidiophores* hyaline, rarely lightly dematiaceous, septate, erect, 28 -290 μm x 2.25 μm wide, branching infrequently, once or twice at most, micronematous. *Conidiogenous cells* holoblastic, apical, integrated, 1 -3, hyaline, 16.5 -49 μm x 2 -2.5 μm, becoming swollen and ridged at the tip with age resulting from a short percurrent proliferation between successive conidia. The conidial primordium is cut off by a septum while still

small and somewhat clavate, extends and becomes curved and septate before dissolution at the basal septum releases the spore. *Mature detached conidia* sigmoid or curved, hyaline, walls thin and smooth, 2 -4 septate, 46 -96 μm x 2.5 -4.5 μm, tapering slightly towards each end, attachment scar flattened, frequently with incipient germ tube protruding through it or laterally from just above it. *Microconidial state* formed under the same conditions though rarely coinciding completely (Fig 3.5L, M, N). *Conidiophores* hyaline or frequently lightly dematiaceous, septate, erect, 0 -500 μm to first phialide x 2 -4 μm wide, rarely branched, micronematous. *Conidiogenous cells* phialidic, 1 -4, apical, on short conidiophores, or borne in groups of 2 -4 laterally on long unbranched verticillate conidiophores, cylindrical or ampulliform, 8 -21 μm x 2 -3 μm, long collarette present (only a little shorter than phialide). *Mature detached conidia* hyaline, walls thin and smooth, rod shaped, cylindrical, with rounded ends, 4.5 -6.5 μm x 0.75 μm.

The shape of the macroconidia and their method of formation from conidiogenous cells which develop annellations at the tip place this fungus close to *Filosporella annelidica*(Shearer and Crane) Crane and Shearer. However, *F. annelidica* has "richly branched" conidiophores (Webster and Descals, 1979) and macroconidia measuring (126-) 164 -208 (-213) x 2 -2.4 μm and 2 -10 septate (Shearer and Crane, 1976). No microconidial state was mentioned in the original description, but Webster and Descals (1979) described an oval phialidic microconidial state from a fungus they later called *F. annelidica?*, and one of the three species placed in synonymy with *F. annelidica*, *Anguillospora virginiana* Wolfe, was originally described as having a similar oval phialospore state (1.5 x 2.5 -5 μm). All three (*A. virginiana*, *A.*

pulchella Wolfe, and *Coeloanguillospora appalachiensis* Dyko and Sutton) were originally described as showing aggregation of conidiophores into sporodochia, synnemata, or cup -shaped fructifications. No such structures were seen in the present fungus. *Filosporella aquatica* Nawawi, the other species in this genus has larger conidia (178 -245 μm x 4 -5 μm, 6 -12 septate), and penicillate conidiophores (Nawawi, 1976). Clearly the present species does not belong to either of these species, but should be considered a new species.

Fontanospora eccentrica (Petersen) Dyko.
Fig. 3.5J, K, Fig. 3.6A, B, C.

Although this species has been previously recorded in New Zealand by Aimer (1982, 1985b), some features worth noting were seen in the present work. Attached conidia were seen in the plating experiment. Fig. 3.5J and K illustrate the relatively short conidiophores and the sympodial proliferation of the conidiogenous cell from slightly below the apex following secession of the first formed conidium, resulting in 2 -3 cylindrical pegs terminating older conidiogenous cells. Detached spores were seen fairly frequently in the leaf bag and stream survey experiments. Where these were abundant a deformed *Tricladium* -like form (Fig. 3.6C) was seen fairly frequently, bearing more than the "superficial resemblance" noted by Descals and Webster (1982b) to *Tricladium patulum*. Only repeated association with typical *F. eccentrica* conidia enabled the aberrant forms to be identified.

Fig. 3.5

Scale 1 = 50 μm.

A -F Attached and detached conidia of *Tumularia aquatica* from isolation agar on which beech leaves from Reid's Ck were plated, except D (from Reid's Ck stream filter).

G,H,I Spores of *Culicidospora aquatica*; G and H from Reid's Ck stream filters, and I from randomly collected leaves from Reid's Ck.

J,K Attached conidia of *Fontanospora eccentrica* from isolation agar following plating of kamahi leaves from Reid's Ck.

L,M,N Microconidia of *Filosporella* sp. from pure culture.

N -W Macroconidia, attached and detached, of *Filosporella* sp. from pure culture.

X,Y,Z Spores of *Gyoerffyella* sp. from fallen beech leaves from Reid's Ck.

a,b Aerial spores of *?Margaritispora aquatica* from kauri leaves from Hakarimata stream.

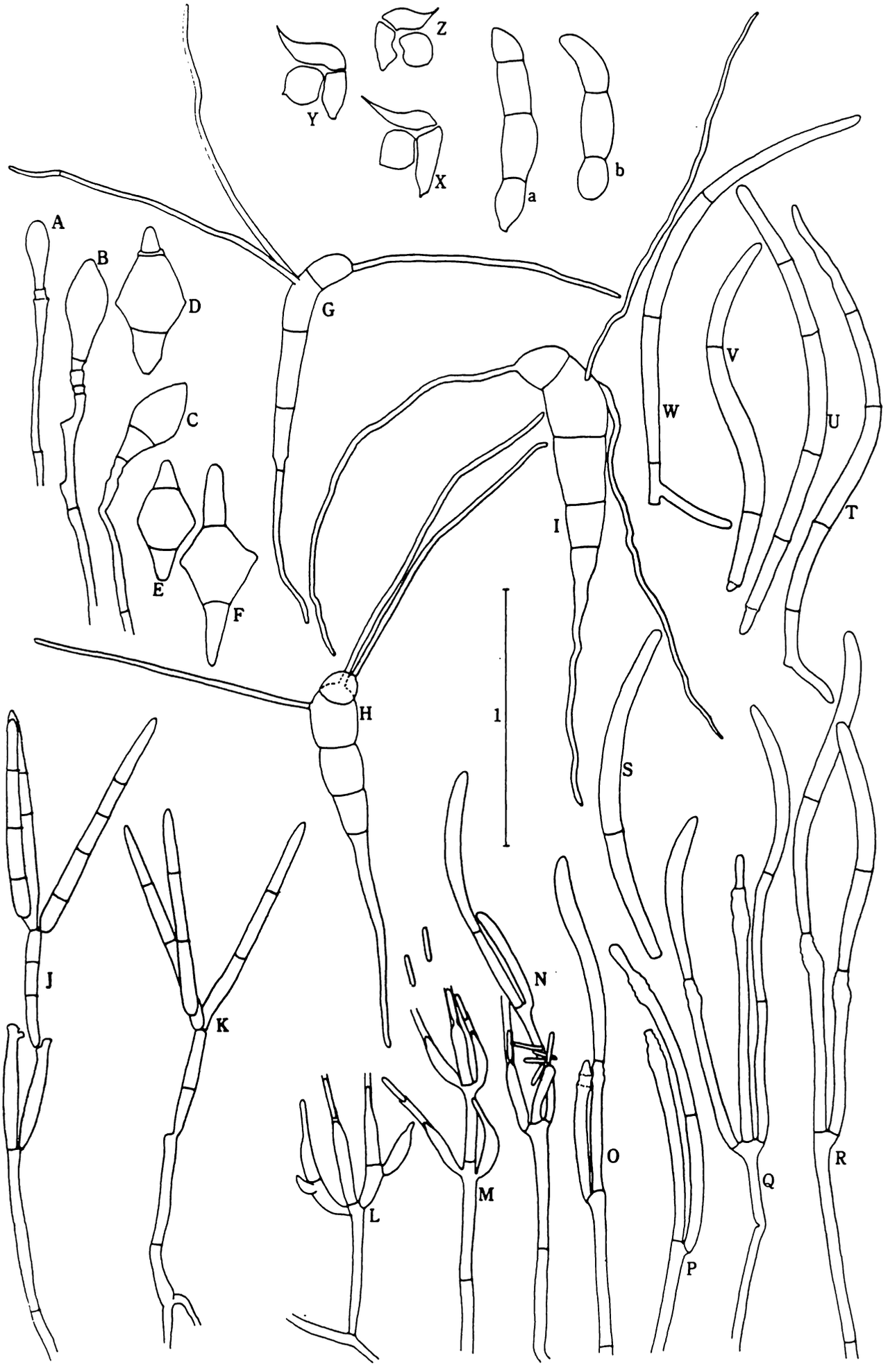

A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X
Y
Z
a
b
1

Fig. 3.6

Scale 1 = 50 μm; Scale 2 = 100 μm.

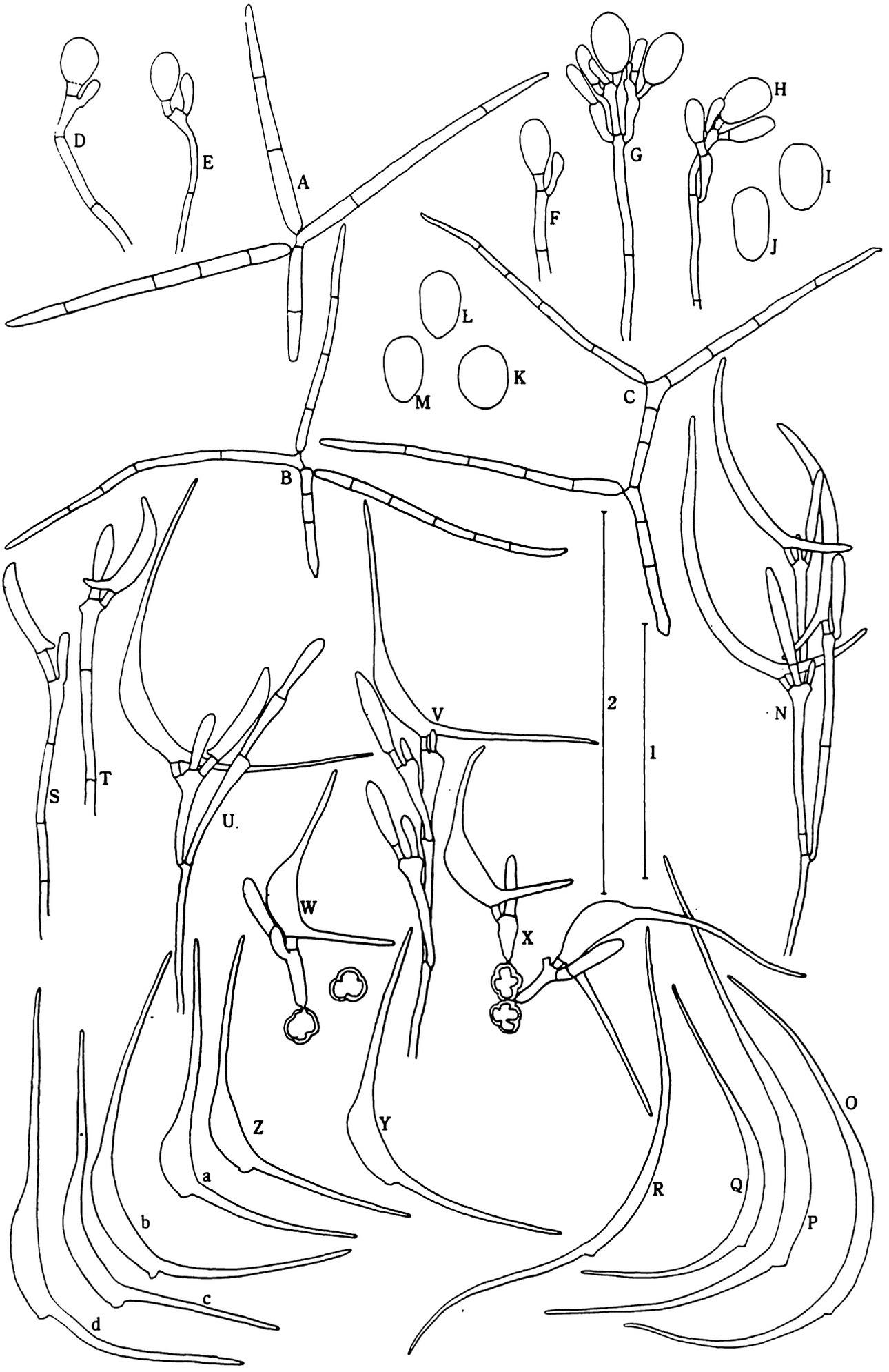
(A,B,C to scale 2, rest to scale 1)

A,B,C Detached spores of *Fontanospora eccentrica* seen on isolation agar following plating of kamahi leaves from Reid's Ck (A) and from Reid's Ck stream filters (B and C), including the *T. patulum* -like form.

D -M Attached and detached conidia of *Lunulospora ovalis* sp. ined. from pure culture.

N -R Attached and detached conidia of *Lunulospora curvula* from New Zealand pure culture (continued in Fig. 7), except P (from Exeter culture).

S -Z and a -d Attached and detached conidia of *Lunulospora cymbiformis*, S -V and Z, a -d from pure culture, and W and X (conidiophores arising from ?chlamydospores) from cultures grown on sterile leaves.


A
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M
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Q
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V
W
X
Y
Z
a
b
c
d
1
2

Fig. 3.7

Scale 1 = 50 μm; Scale 2 = 100 μm; Scale 3 = 200 μm.

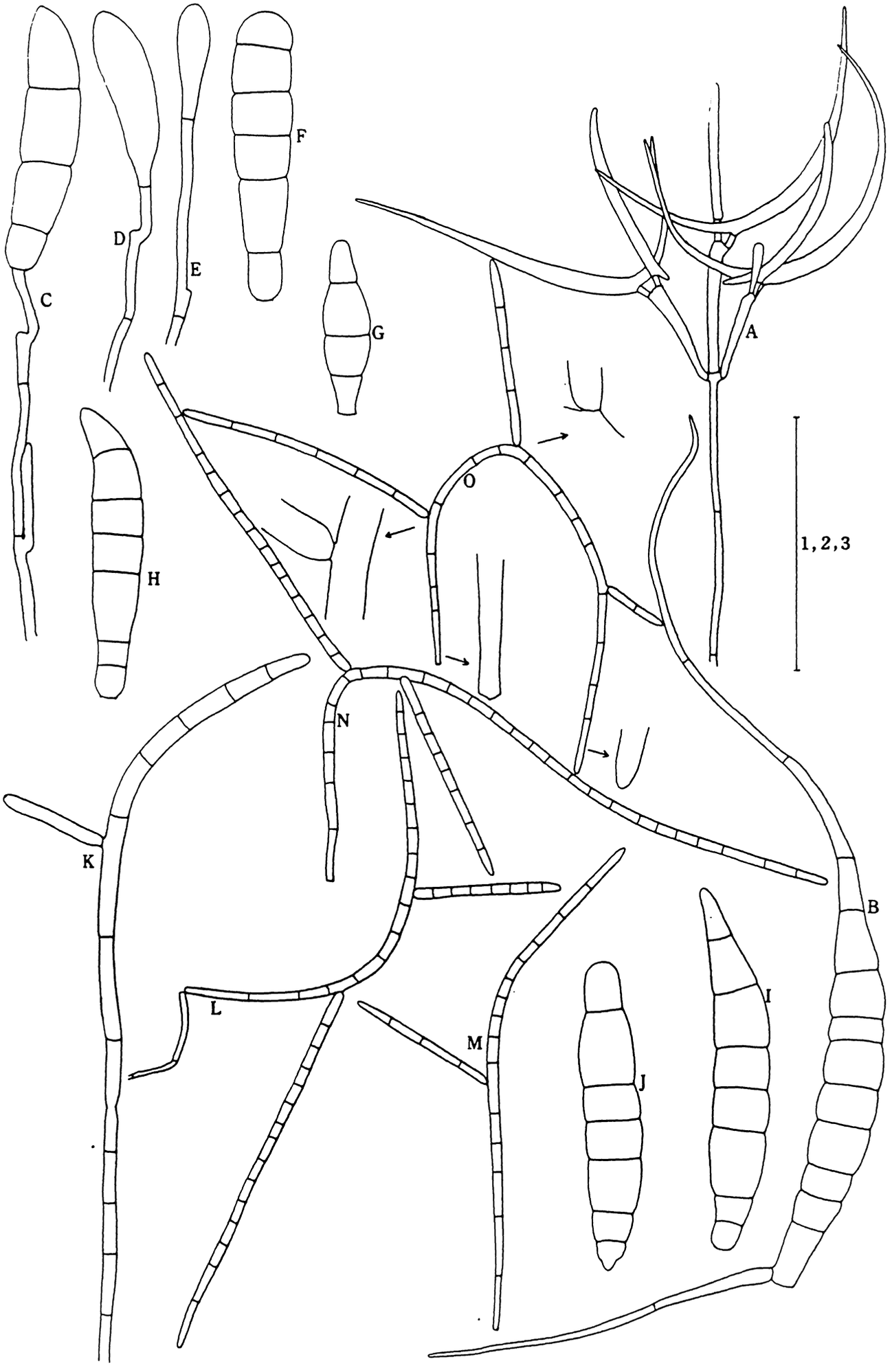
(A, C -J, and details of O scale 1; B,K scale 2; L,M,N,O scale 3)

A *L. curvula* continued.

B Detached spore of *Mycocentrospora acerina* from isolation agar on which beech leaves from Reid's Ck were plated.

C -J Attached and detached conidia of *Mycocentrospora clavata* from isolation agar on which kamahi leaves from Hakarimata stream were plated.

K -O Attached and detached conidia of *Tricladium* sp. from isolation agar following plating of kamahi leaves from Hakarimata stream. (Includes details of branch junctions, apex, and attachment point of spore "O").


A
B
C
D
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F
G
H
I
J
K
L
M
N
O
1, 2, 3

Gyoerffyella sp.

Fig. 3.5X, Y, Z.

This species was seen only a few times, as detached spores on filters, where the cell walls can be difficult to make out. However, the spores fairly clearly consist of a roughly obclavate, basal attachment cell, the attachment point sometimes flattened or apiculate, and a tapering apical cell, curving about and then away from the attachment point of an inflated branch cell arising from just below the septum dividing the basal and apical cells. The curved, tapering axis, curled about the origin of a lateral branch is clearly reminiscent of *Gyoerffyella*, particularly the simplest species, *G. entomobryoides* (Boerema and Arx) Marvanová, but the spores do not correspond to any published species.

Heliscella ?stellatacula (Kirk ex Marvanová and S. Nilsson) Marvanová

Fig. 3.4O, P, Q, R.

Spores such as these were seen infrequently in the leaf bag and stream survey experiments, but could have been missed sometimes on low power counts because of their small size. They clearly belong to either *H. stellatacula* or *H. stellata* (Ingold et Cox) Marvanová. Similar spores were recorded in New Zealand streams by Aimer and Segedin (1985b). The problem with identifying these lay with drawings in Marvanová (1980) which gave conidium lengths of 12 -14 μm for *H. stellatacula* and c. 20 μm for *H. stellata*. However, comparing these measurements with Nawawi (1985) and the original descriptions it is apparent that the scale for E and F in Fig. 2 and 3 respectively in

Marvanová (1980) should read 10 μm not 20 μm. The two species may also differ in habitat, *H. stellatacula* having been originally described from an estuary, although according to Marvanová (1980) it is salt tolerant only and does not sporulate as freely in artificial sea water as in distilled water. According to size, therefore, it appears the present spores (spore length 5 -7 μm) are most likely to belong to *H. stellatacula*.

Lunulospora spp.

There are two current species in the genus, *L. curvula* Ingold and *L. cymbiformis* Miura. *L. curvula* has been previously described from a New Zealand isolate, and *L. cymbiformis* from detached spores Aimer and Segedin (1985b). Both species were isolated several times from the Hakarimata stream in the course of the present work, enabling a detailed comparison to be made. The only previous description of *L. cymbiformis* is the original one (Miura, 1972).

L. cymbiformis

Fig. 3.6S -Z and a -d.

Colony growth rate 0.80 mm/day; colony margin hyaline, darkening to pale olive before being covered by short cream aerial mycelium which itself darkens with age, sometimes quite abruptly, to brownish -grey; reverse shades from hyaline margin through striated olive to dark olive; submerged hyphae hyaline, septate, branching, c.2 μm diam. *Conidiophores* hyaline, septate, erect, simple or 1 -3 branched, 95 - 450

x 2 -2.5 μm to first branch, micronematous. *Conidiogenous cells* holoblastic, apical, 1 -4, hyaline, becoming swollen at the apex with successive sympodial and percurrent proliferations, 10 -44 x 2.5 -3 μm, swelling to 6.75 μm, up to 3 conidia developing concurrently. The conidial primordium develops as a finger -like projection which elongates and is cut off by a basal septum. A second septum forming a short distance above delimits a short separating cell (2.5 -6 (-10) x 1.25 -2 μm) from the conidial primordium proper. Following the formation of this second septum the primordium elongates at the apex, and retrorsely from just beside the attachment point, both extensions tapering from the wider middle portion. The conidium becomes curved or bent away from the point of attachment and is eventually released by breakdown of the separating cell. *Mature detached conidia* hyaline, walls thin and smooth, curved, sometimes uniformly but more often bent sharply at a point opposite the attachment scar, which is prominent and located a short distance below the middle of the conidium. Conidia are commonly, but not invariably, somewhat inflated at the widest point, just above the attachment scar, but at 13 -23 μm above it the conidium tapers rapidly over a short distance to the width of the conidium below the attachment scar (1.75 -2.5 μm). Both the apical process and lower part of the conidium (basal process) taper gradually throughout their length. Conidia 42.5 -115 x 3 -6.25 μm at widest point.

The description agrees well with that of Miura (1972). In addition chlamydospore -like structures were seen in water filtered off sterile leaves that had been inoculated with *L. cymbiformis*. These were giving rise directly to conidiogenous cells bearing conidia (Fig. 3.6W, X).

L. curvula

Fig. 3.6N -R.

The extra isolates of *L. curvula* obtained in the present work differed from the isolate described by Aimer and Segedin (1985b) only in the slightly greater range of conidial measurements and somewhat greener colony colour where not covered in the sparse grey aerial mycelium, with the reverse hyaline shading into very dark bottle green or black. Submerged hyphae measured 2.5 μm in diameter and were very lightly dematiaceous in older parts, conidiophores measured 87.5 -550 x 1.5 -2.5 μm and were 0 -1 branched, conidiogenous cells were 13.75 -35 x 2 -2.5 μm swelling to 4.5 μm, up to 3 per branch, separating cells were 2.5 -4.5 x 1 -2 μm, and mature detached conidia measured 72.5 -127.5 x 2.5 -3.75 μm. The growth rate recorded in Aimer and Segedin (1985b) is a misprint and should read 1.04 mm/day at 10 C. The newer isolates averaged 1.20 mm/day at 13 C on 1.5 % MA.

An English isolate of *L. curvula* was obtained from Exeter University and compared with the New Zealand isolates. There were a few small differences only: shorter conidiophores (30 -125 μm), only 1 -2 conidiogenous cells per conidiophore branch, and conidia up to 5 μm wide. Much of the mycelium on mature parts of the colony was irregularly swollen and this, together with the differences outlined may indicate only that it is an old isolate. The differences are not considered significant.

A comparison of the two species reveals two main differences, one being colony morphology and growth rate on agar, and the other conidium morphology. *L. cymbiformis* is slower growing (0.80 c.f. 1.20 mm/day), less green in colour and has more aerial mycelium. The species are thus easily distinguished in pure culture. Conidia of *L. curvula* taper evenly throughout their length, being curved or sigmoid rather than

bent, and are commonly narrower than *L. cymbiformis* (2.5 -3.75 (-5) compared with 3 -6.25 μm). However, there is considerable overlap and large numbers of apparently intermediate forms are seen in ecological work using detached spores. Hence all detached spores were counted as "*Lunulospora* spp." in most of the present work, even though *L. cymbiformis* appeared to dominate in most collections.

L. ovalis sp. ined.

Fig. 3.6D -M.

This species has been previously described by Aimer and Segedin (1985b) as "*D.* aff. *foliicola*". A few further illustrations only are given here.

The separating cell in "*D.* aff. *foliicola*" is like that of *Lunulospora* rather than, for example, *Anguillospora*, in that on secession the cell vanishes leaving no trace on either conidium or conidiophore. The fate of this cell requires more work. However, its shape, size, and apparent action appear closely similar to that of published *Lunulospora* species. "*D.* aff. *foliicola*" resembles *Lunulospora* also in the structure of the conidiophore and its method of proliferation, and in the dark colour and limited aerial mycelium of the colony on agar. It seems likely the two fungi are closely related. It is clear that *D.* aff. *foliicola* and *D. foliicola* are not closely related, the only similarity really being the superficial resemblance between their oval conidia. The difference in secession is particularly significant, and for this reason it is suggested that the species be placed in a separate genus, that of *Lunulospora*, following modification

of the definition of *Lunulospora* conidium shape. The name *Lunulospora ovalis* is proposed, based on the most distinguishing feature of this species within the genus, that of conidium shape. Within the genus conidium shape can be envisaged to follow a progression from *L. curvula* to *L. cymbiformis* with the development of the central inflated part, and then to *L. ovalis* in which the attenuated portions have been lost and only the inflated part remains.

Margaritispora aquatica Ingold

Fig. 3.5a, b.

Recent work by Marvanová and Descals (1985) indicates that the spores depicted from New Zealand by Aimer and Segedin (1985b) are more likely to belong to *M. aquatica* than *M. monticola* Dyko (now *Goniopila monticola* (Dyko) Marvanová & Descals). This is because *M. aquatica* has conidiophores like the fragment depicted. In the course of the present leaf bag experiment two spores were seen that were very similar to *M. aquatica* aerial spores.

Mycocentrospora acerina (Hartig) Deighton.

Fig. 3.7B.

This species was seen several times in the course of the plating experiment. The spores are very distinctive. The species is unusual among aquatic hyphomycetes in that it is also an important pathogen on a number of crops such as carrot, and has also been reported as a human pathogen (Deighton and Mulder, 1977).

Mycocentrospora clavata Iqbal.

Fig. 3.7C -J.

This species was seen in the plating experiment, from which the following incomplete description was obtained.

Conidiophores hyaline, erect, septate, occasionally branched, 2 -2.5 μm wide. *Conidiogenous cells* holoblastic, apical, integrated, single, hyaline, proliferating sympodially about conspicuous attachment scars of previous conidia, bearing a single apical conidium. The clavate conidial primordium is cut off from the conidiogenous cell, elongates, becomes septate and swollen and is shed by disarticulation at the basal septum. *Mature detached conidia* hyaline, walls thin and smooth, fusiform to clavate, straight to slightly curved in usually the anterior quarter or half of the spore, attachment scar flattened, conspicuous, with occasional proliferation through it, basal cell narrows abruptly to attachment scar, 34 -71 x 9.5 -11.5 μm tapering to 2.5 -3 μm at the attachment scar, 3 -7 septate, irregularly spaced, cells frequently inflated, stain often denser in basal and apical cells but smooth in all.

The broad conspicuous attachment scars and sympodial proliferation of relatively simple conidiophores places this fungus in *Mycocentrospora*. *Mycocentrospora clavata* is the most closely similar species, the only difference being slightly larger conidia (55 -88 x 10 -13 μm) (Iqbal, 1974). The conidia of *Colispora elongata* Marvanová are also closely similar to the present fungus, but differ in their formation: conidiophores proliferate percurrently compared with sympodially, as in the present case (Marvanová, 1988).

Tetracladium sp.

Fig. 3.4S -Z and a -c.

This species was isolated from randomly collected leaves from Hakarimata str. The following incomplete description is from slides only as the culture was not kept.

Conidiophores hyaline, erect, septa present or absent, simple or irregularly 1 -4 branched, 10 -75 x 1.5 -2 μm, micronematous. *Conidiogenous cells* holoblastic, apical, single, integrated, quite indistinguishable from conidiophore generally. Conidial primordia arise as finger -like growths from the apex, up to 3 at a time, developing in succession. *Mature detached conidia* hyaline, walls thin and smooth, tetraradiate, consisting of a main, attachment, axis which gives rise to 3 arms in one plane, like the prongs of a fork; one of the lateral arms frequently, and the central arm usually, are blunt ended, finger -like, and have a tapering branchlet budded from just below the terminal cell; the third arm is simple; main axis 6.5 -11.5 x 2.5 -3 μm, tapering to the narrow attachment point, 0 -1 septate, branched arms 5.5 -11 x 2.5 -4 μm, 0 -2 septate, branchlets 3 -7 x 2 -3 μm, simple arm(s) 5 -15 x 2 -3 μm, 0 -1 septate.

The conidium form is typical of *Tetracladium*, with the angle of divergence of the lateral arms initially 90 ° but almost immediately turning upwards to 45 - 60 ° (sometimes diverging further gradually after this). It does not match any published species however. Of the species possessing digitate arms the conidium size is closest to that of *T. apiense* Sinclair and Eicker, but this species has 4 arms. The simple arms taper less gradually in *T. apiense* than in the present species generally, and the arm orientation is less typical of *Tetracladium* as

described above. *T. setigerum* (Grove) Ingold and *T. furcatum* Descals both have 4 arms also, and are significantly larger than the present species. In *T. maxilliformis* (Rostrup) Ingold the conidia are less branched than in the present fungus, the attachment arm shorter, the branchlet equivalent more finger -like, and the simple arms more tapered and whip -like.

Tricellula aquatica Webster

Fig. 3.4d -j.

This species was isolated from randomly collected leaves from the Hakarimata stream, and is a new record for New Zealand.

Colony growth rate 0.34 mm/day at 13 C; apricot coloured, slightly paler towards margin, flat and wet looking, no aerial mycelium, reverse cream shading into soft apricot; submerged hyphae hyaline, septate, branching, up to 2 μm diam. *Conidiophores* absent or very short and simple, lateral, hyaline, erect, consisting of 1 or few commonly clavate cells, 3 -6 x 1.5 -3 μm to first conidiogenous cell. *Conidiogenous cells* holoblastic, sessile on vegetative hyphae or apical, occasionally lateral, 1 -several, on short conidiophore, hyaline, 3 -6.25 x 1.5 -3 μm, fusiform to clavate, frequently swollen to ellipsoidal, bearing several conidia simultaneously, successive conidia accumulating in large clumps about the conidiogenous cell. Conidial primordia are budded off the tip of the conidiogenous cell, elongate and swell to about final size before budding off two arms in succession in a similar manner. Conidium release is by basal constriction. *Mature detached conidia*

hyaline, walls thin and smooth, triradiate, consisting of a clavate basal cell with two fusiform to obclavate arms radiating antrorsely at a narrow angle, arms constricted at the base and frequently uneven in length, occasionally 1 septate and constricted at the septum, total spore length 6.25 -11.25 (-17.25) μm, basal cell 1.25 -2.5 μm wide, branches 1 -2.5 μm wide.

Tricladium sp.

Fig. 3.7K -O.

This species was seen only in the course of the plating experiment, from which the following partial description was obtained.

Conidiophores hyaline, erect septate, unbranched, 182 -858 μm, width decreasing away from the conidium to 1.5 μm, micronematous. *Conidiogenous cells* holoblastic, apical, single, integrated, 5 μm diam., no evidence of proliferation seen. The conidial primordium commences as a finger -like extension of the conidiophore apex which elongates, becomes septate, and buds out 1 -4 branches from along its length. Finally a basal septum forms and conidium release occurs through disarticulation at this septum. *Mature detached conidia* hyaline, walls smooth and thin, consisting of a curved, septate axis with 1 -4, most commonly 2, branches, constricted at the origins, septate, arising at intervals usually from the same side of the axis and radiating perpendicularly or at a slightly acute angle away from it, attachment scar flat, apices acute, axis 390 -624 x 7 -9 μm, tapering slightly towards base and apex to 3.5 -6 μm, branches 26 -338 x 6.5 -8 μm, tapering to 5 μm, successive branches decreasing in length and, slightly, in width.

This fungus appears to be a species of *Tricladium*, with conspicuous similarities to the type species *T. splendens*. However it does not correspond to any published species either in its large size or constricted branch origins. Tubaki (1966) described a *Varicosporium* state for *Hymenoscyphus varicosporoides* Tubaki which corresponds in size (main axis 300 -500 x 7 -7.5 μm) and has constricted branch origins. It was placed in *Varicosporium* because secondary branches are produced regularly, which the present fungus does not. *Tricladium* was considered, as the conidia consisted of an axis and one or two laterals "in the early stage", secondary branches on laterals developing "when mature", which may mean after release. If this is the case it is perhaps possible the present fungus is conspecific with Tubaki's, but that the spores were not old enough for secondary branching to have occurred. Nawawi (1985) noted considerable variation in branching and that spores without secondary branching are *Tricladium* -like. Descals and Webster (1982b) suggested further work was needed on the species as conidium ontogeny was not clear.

Tumularia aquatica (Ingold) Descals & Marvanová.
Fig. 3.5A -F.

Spores of this species were seen occasionally in the leaf bag and stream survey experiments, and were seen attached in the plating experiment but it was not isolated. It is a new record for New Zealand.

Mature detached conidia are hyaline, walls smooth and thin, roughly lemon shaped, 2 septate, the septa dividing the spore unequally into a large swollen middle cell and smaller apical and basal cells, spore size

28.5 -31 (-52) x 13.5 -16 (-19) μm. The apical and basal cells may become more elongated in older spores, which also develope small tubercles about the widest point of the middle cell (Fig. 3.5D).

Unidentified species 1

Fig. 3.4E -N.

This species appeared regularly in the Hakarimata stream in the leaf bag experiment in low numbers, and in the plating experiment, from which the following incomplete description is taken.

Conidiophores hyaline, erect, septate, branching irregularly, short, c. 2.5 -3 μm diam., micronematous. *Conidiogenous cells* phialidic, apical, usually single, integrated, hyaline, fusiform, 15 -22.5 x 2 -2.5 μm, bearing a single terminal conidium, rarely lateral, collarette present though not always visible. *Mature detached conidia* hyaline, walls thin and smooth, oval to cylindrical, occasionally slightly obovoid, attachment point not conspicuous, 10.5 -15 x 3 -6 μm, cytoplasm has a granular appearance when stained.

Another fungus was seen with conidia rather similar in appearance in the plating experiment, but was easily distinguished through being slightly smaller (8 x 2.5 μm) and not phialidic. The present species is somewhat like *Cladobotryum* but lacks the verticillately branched conidiophores found in this genus. *Phialophora* is another possible genus which might accommodate the fungus, but most species have pigmented phialides, prominent collarettes, and much smaller conidia. Many species of *Cylindrocladium* have microconidial states which are much more like the present species. For example, *C. cylindroides* Wollenweber

has oval to cylindrical microconidia, 8 -14 x 4 -6 μm, formed from loosely branched conidiophores terminating in single phialides, and "most isolates...show a reluctance to form macroconidia in culture. Isolates are most easily identified on the size of the microconidia." (Booth, 1966). This species is recorded as parasitic on Pinaceae in North America and Europe.

Unidentified species 2

Fig. 3.3T -Z.

This species was distinguished in the plating experiment from leaves from Reid's Ck. The conidia are very similar in appearance to those of *Dimorphospora foliicola* and *L. ovalis* sp. ined., which are, fortunately, very rare (seen only once in the plating experiments) or apparently absent respectively in Reid's Ck and Hakarimata.

Conidiophores hyaline, erect, septate, simple, 1 -1.25 μm diam., micronematous. *Conidiogenous cells* polyblastic, apical, subapical or occasionally lateral, up to 3 conidiogenous loci per conidiophore, separated by up to 25 μm, integrated, hyaline, fusiform to spherical, more normally swollen, to 3.25 μm wide, proliferation percurrent or sympodial. Conidia begin as small buds distributed over the surface of the conidiogenous locus, and appear to mature one at a time, swelling to a spherical shape attached by a narrow isthmus. *Mature detached conidia* hyaline, walls thin and smooth, broadly ellipsoidal to spherical, rarely with attachment peg remaining, 8 -10 x 7.5 -8.5 μm, stain smooth and pearly, a darker patch normally visible at conidial poles.

The present species might belong in *Blastobotrys*, although no fragmentation of conidiophores was seen, and the conidiogenous loci in *Blastobotrys* are more often lateral on the conidiophore than integral as in the present species. Published species have much smaller conidia than the present species (Sesma and Ramirez, 1978). In *Gonatobotrys* conidia arise simultaneously on short denticles over the surface of terminal and node -like swellings on the conidiophore. In the present fungus conidia may arise simultaneously, but appear to mature in succession rather than simultaneously as in *Gonatobotrys*. Conidia of *G. simplex* Corda, the closest species, have more conspicuous attachment points and are perhaps less spherical than the present species. Thus the present fungus is most likely to be an unnamed species of *Gonatobotrys*.

Unidentified sp. 3.

Fig. 3.9K, L.

This fungus was seen only as detached spores in the leaf bag experiment, but was prominent on rimu and ponga.

Unidentified sp. 4.

Fig. 3.10T -Y.

This fungus was also seen only as detached spores in the leaf bag experiment, but was prominent late in decay on some leaves in the leaf bag experiment.

Fig. 3.8

Scale 1 = 50 μm.

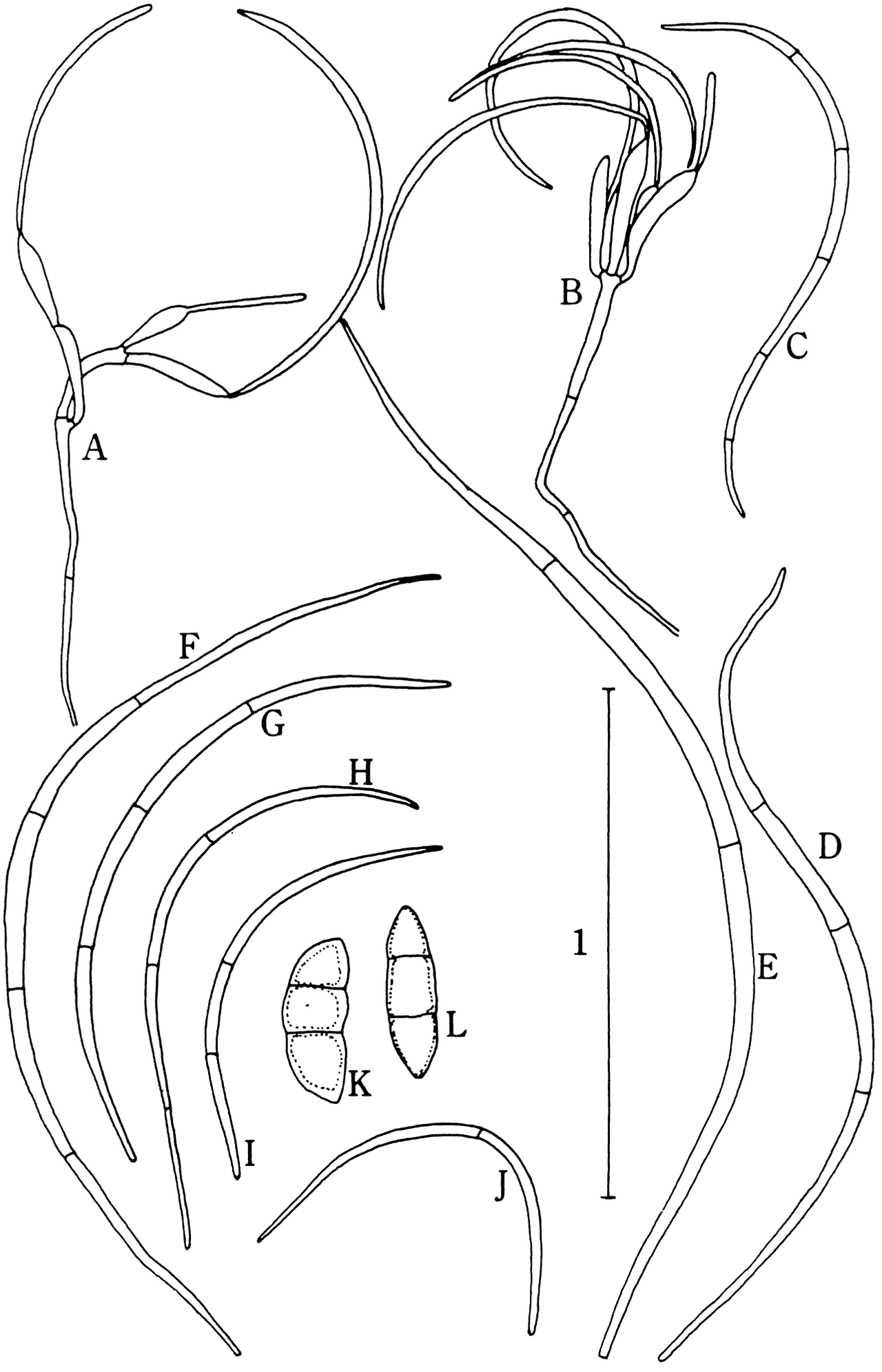
A -J Unidentified sigmoid/rod type 1:

A,B,C Attached and detached conidia from isolation agar on which kamahi leaves from Reid's Ck were plated.

D,E,F Detached spores from leaves (D,F kamahi, E random) from Reid's Ck.

G,H,I,J Detached spores from leaves and stream filters from Reid's Ck (G,H stream filters).

K,L Thick walled detached spores of Unidentified sp. 3 from ponga leaves.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w,x,y Detached conidia of Unidentified sigmoid/rod type 8 from isolation agar on which beech leaves from Hakarimata were plated (also seen attached -phialidic).

z,aa,bb,cc,dd Attached and detached conidia of Unidentified sigmoid/rod type 9 from isolation agar following plating of kamahi leaves from Hakarimata.

Fig. 3.9

Scale 1 = 50 μm; Scale 2 = 100 μm.

(All scale 1 except o,p,q,r)

A -F Attached and detached conidia of Unidentified sigmoid/rod type 2, A -D from isolation agar on which kamahi leaves from Hakarimata stream were plated, E from willow leaves from Firewood Ck, and F from kamahi leaves from Reid's Ck.

G,H,I Detached spores of Unidentified sigmoid/rod type 3 from isolation agar on which beech leaves from Reid's Ck were plated.

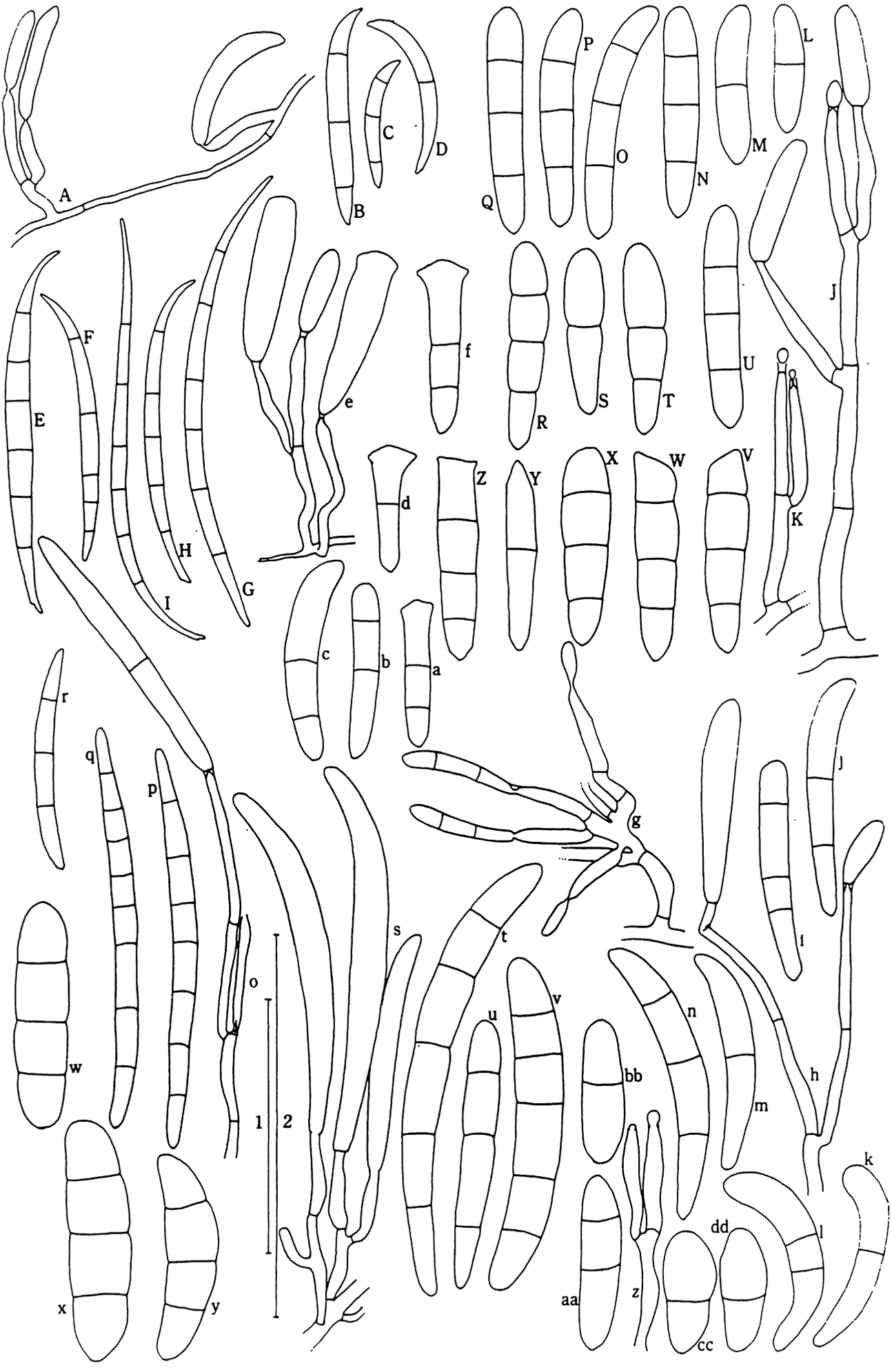
J -U Attached and detached conidia of Unidentified sigmoid/rod type 4, J -Q and U from pure culture, R,S,T from isolation agar following plating of kamahi leaves from Hakarimata stream.

V -Z and a -f Attached and detached conidia of *Heliscus lugdunensis* from isolation agar following plating of beech leaves from Reid's Ck (X,b,c are probably contaminant Unidentified sigmoid/rod type 4 or 6, but could possibly represent deformed spores without arms altogether, as V,W and Y are reduced to only one arm).

g -n Attached and detached conidia of Unidentified sigmoid/rod type 6, g -l from isolation agar on which kamahi leaves from Reid's Ck were plated, m and n from that on which beech leaves from Hakarimata were plated.

o,p,q,r Attached and detached conidia of Unidentified sigmoid/rod type 5 from isolation agar on which beech leaves from Reid's Ck were plated.

s,t,u,v Attached and detached conidia of sigmoid/rod type 7 from isolation agar following plating of kamahi leaves from Hakarimata.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Unidentified sigmoid/rod type 1

Fig. 3.8A -J.

There are probably several species shown here from the leaf bag and plating experiments. The drawings can be divided into 3 groups:

A) small sigmoid conidia, seen in the plating experiment (Fig. 3.8A, B, C) which develop from small groups of phialides (7 -14 x 1.5 -2 μm), terminal on conidiophores of 1.5 μm diam. The conidia measure c. 60 x 1.5 μm, have several septa, and taper acutely.

B) slightly larger sigmoid conidia (Fig. 3.8D, E, F, G), which frequently have a flattened attachment point, measure 60 -115 x 1.5 -2 μm, and have 2 -3 septa only. These were seen in the leaf bag experiment as detached conidia only and were counted as Unidentified sigmoid/rod type 1(2).

C) small thin sigmoid spores seen in the leaf bag experiment also as detached spores only (Fig. 3.8H, I, J). They measure 34 -73 (-90) x 1 -<1.5 μm, have 1 -several (more rarely) septa, and taper to acute ends. These spores themselves may belong to more than one species, and some may belong to the same species as type A. They were counted as Unidentified sigmoid/rod type 1(3).

Flagellospora has sigmoid phialospores, but all published species are wider and generally longer than the present type(A). *F. curvula* Ingold has conidia of 100 -150 x c. 2 μm, distinctly sigmoid or curved,and tapering to 1.5 μm. Type B could thus belong here but not type A or C. Marvanová and Descals (1985) describe some isolates of *Alatospora acuminata* as producing mostly acicular conidia, up to 60 μm long and 1 -2.5 μm wide, 1 -6 septate, tapering, and curved or sigmoid. The conidiophores are 15 -20 x 1.5 -2 μm, with 1 -4 terminal or lateral phialides, 8 -15 x 2 -2.5 μm. This description fits type A well. The spores recorded in type C could belong to a wide range of species in the

Hyphomycetes, Coelomycetes, or even Ascomycotina, as well as, perhaps, *A. acuminata*. No further identification can be attempted without attached spores.

Unidentified sigmoid/rod type 2

Fig. 3.9A -F.

Conidia referable to *Fusarium*, that is slightly curved, fusoid, septate conidia with a more or less conspicuous foot cell, were seen regularly in small numbers in the leaf bag experiment and stream survey. Two other spores also possibly belong to *Fusarium*, or perhaps *Flagellospora* in the case of type 3:

Unidentified sigmoid/rod type 3 (Fig. 3.9G, H, I)

Unidentified sigmoid/rod type 13 (Fig. 3.12H, I).

Unidentified sigmoid/rod type 4

Fig. 3.9J -U.

This species was isolated during the plating experiment, but was not kept to describe fully. A partial description of this isolate follows (Fig. 3.9J -Q, U).

Colony not described. Dematiaceous chlamydospores present, 4.5 -9 μm diam. *Conidiophores* hyaline, erect, septate, irregularly branched, up to 5 branches seen, 37.5 -250 x c. 3 μm to first branch, branches 27.5 -180 μm, micronematous. *Conidiogenous cells* phialidic, apical, 1 -3, integrated, hyaline, fusiform, tapering slightly towards apex, 17.5 -37.5 x 3 μm, collarette present though not always clear. *Mature detached conidia* hyaline, walls thin and smooth, rod shaped, sometimes

curved slightly (anterior part only), cylindrical to slightly clavate, attachment end tapered, anterior end rounded, (21)25 -48 x (5)6 -7 μm, 0 -3 septate.

This isolate almost certainly belongs to the genus *Cylindrocarpon*, some species of which have been previously recorded from submerged habitats. However, it does not correspond exactly to any published species. *C. obtusisporum* (Cooke and Harkness) Wollenweber has been previously recorded from New Zealand on *Beilschmedia* and *Coprosma*. It appears to be closely similar to the present isolate, but differs in the presence of a microconidial state. Two other species are also closely similar. *C. olidum* (Wollenweber) Wollenweber has been recorded from crops such as asparagus and pears but differs in physical characteristics only in the greater range of conidium size and septation reported. *C. destructans* (Zins.) Scholten occurs in both pathogenic and saprophytic strains and is widespread in forest soils. It also differs in possessing a somewhat greater range of conidium size and septation, and in addition has a microconidial state not observed in the present fungus.

What appeared to be the same species was seen numerous times in the plating experiment as attached and detached conidia (Fig. 3.9R, S, T). Quite frequently the conidial cells were somewhat inflated, with diameters up to 8.5 μm measured, and conidia up to 57 μm long. Similar spores were seen only rarely in the leaf bag experiment. Although recorded as type 4 they cannot be certainly identified as the same species because there are a large number of genera and species which produce rod -shaped septate spores (Nilsson, 1964). *H. lugdunensis* Saccardo and Therry in particular appears to be closely related to *Cylindrocarpon* with septate macroconidia varying in shape from "cylindrical -clavate" to "clove -shaped" (Webster, 1959). A variety of

conidia belonging to this species were also seen in the plating experiment (Fig. 3.8V -Z and a -f), from which the difficulty of distinguishing the two species when only one or a few detached spores are present can be seen.

Unidentified sigmoid/rod type 12

Fig. 3.10A -F.

Fungi with septate, rod -shaped but markedly smaller conidia than the above type 4 were seen numerous times in the plating experiment. Where these were seen attached some attempt to identify them could be made. On some occasions the fungi were clearly phialidic (Fig. 3.10A, G), while in others whether conidiogenesis was phialidic was unclear (Fig. 3.10K). The phialidic form(s) at least probably belong to *Cylindrocarpon*, as there are several small spored species, for example, *C. tenue* Bugn., *C. gracile* Bugn., *C. magnusianum* Wollenweber, *C. orthosporum* (Saccardo) Wollenweber, and *C. aquaticum* (Nilsson) Marvanová and Descals. *C. tenue* and *C. gracile* are both only 0 -1 septate compared to the present 0 -3 septate conidia. *C. magnusianum* and *C. orthosporum* correspond well with the smaller sized spores seen particularly (Fig. 3.10A -F), fitting most exactly with *C. orthosporum*. However, *C. orthosporum* is parasitic whereas *C. magnusianum* is reported as a soil saprophyte. *C. aquaticum* is the most likely identification, as this species was originally described from streams, and its conidium size range (15 -53 x 3.0 -5.3 μm) and septation (1 -3(-4)) correspond well with that of the present spores (15.5 -50 x 2.5 -4 μm, 0 -3 (-5)). A definite identification is limited mainly by the small amount of

material observed, which does not all necessarily belong to the same species.

Smaller phialidic 2 -celled conidia like those of *C. tenue* and *C. gracile* were seen, but these clearly belonged to *Cylindrocladium*, probably *C. parvum* on the basis of conidium length and septation, and all 0 -1 septate rods were counted simply as "small rods" in the plating experiment.

Multiseptate rods similar in size to those seen in the plating experiment were seen in the leaf bag experiment, as detached spores only. These could belong to any of a large number of genera (Nilsson, 1964), including *Cylindrocladium* or *Cylindrocarpon*. Unidentified sigmoid/rod type 12 was designated to hold all rod -like spores of dimensions 9 -48 x 2 -4 μm and 1 -5 septate, usually, but not necessarily, with a tapering attachment scar, and more rounded apex.

Several other spore types probably belonged to *Cylindrocarpon*:

Unidentified sigmoid/rod type 5 (Figs. 3.8o -r)

Unidentified sigmoid/rod type 6 (Fig. 3.8g -n)

Unidentified sigmoid/rod type 7 (Fig. 3.8s -v)

Unidentified sigmoid/rod type 8 (Fig. 3.8w, x, y)

Unidentified sigmoid/rod type 9 (Fig. 3.8z, aa -dd).

Unidentified sigmoid/rod type 10 (Fig. 3.12A -E) was also similar, but could have belonged to any of a number of genera including *Cylindrocarpon* as the spores were only seen detached.

Unidentified sigmoid/rod type 14

Fig. 3.12J -T.

Flagellospora penicillioides Ingold, previously recorded in New Zealand by Aimer and Segedin (1985b), was isolated several times in the plating experiment and detached conidia seen regularly in the leaf bag experiment (Fig. 3.12a -d). Some care was needed in the identification of this species. Where conidia were abundant even inflated, aged conidia could be confidently identified as *F. penicillioides* (Fig. 3.12d). However, several slightly different conidial types were seen in the plating and leaf bag experiment, which could not be so confidently identified. It is possible some or all of these may represent distorted *F. penicillioides*, but the differences seen in some conidia, such as a roughly flattened attachment point, combined with greater conidium curvature, septation, and blunter apex (Fig. 3.12N -T), ought to be considered significant. The few attached conidia seen (Fig. 3.12J -M) were phialidic, but the phialides were not in groups of three as is typical of *F. penicillioides* even when the conidiophore branching is reduced (Fig. 3.12a). Unidentified sigmoid/rod type 14 was designated to accommodate these somewhat *F. penicillioides* -like conidial types, whether or not they are all the same species.

Other small spores superficially somewhat similar were:

Unidentified sigmoid/rod type 15 (Fig. 3.12U, V, W)

Unidentified sigmoid/rod type 16 (Fig. 3.12e -j)

Unidentified sigmoid/rod type 17 (Fig. 3.12X, Y, Z).

Fig. 3.10

Scale 1 = 50 μm.

A -O Attached and detached conidia of Unidentified sigmoid/rod type 12, A -F and G -J from isolation agar following plating of kamahi leaves from Reid's Ck, K -M following plating of kamahi leaves from Hakarimata.

P,Q Unidentified spores from kauri leaves (Hakarimata).

R,S Unidentified spores from ponga leaves (Hakarimata).

T -Y Detached spores of Unidentified sp. 4 from kamahi leaves from Reid's Ck.

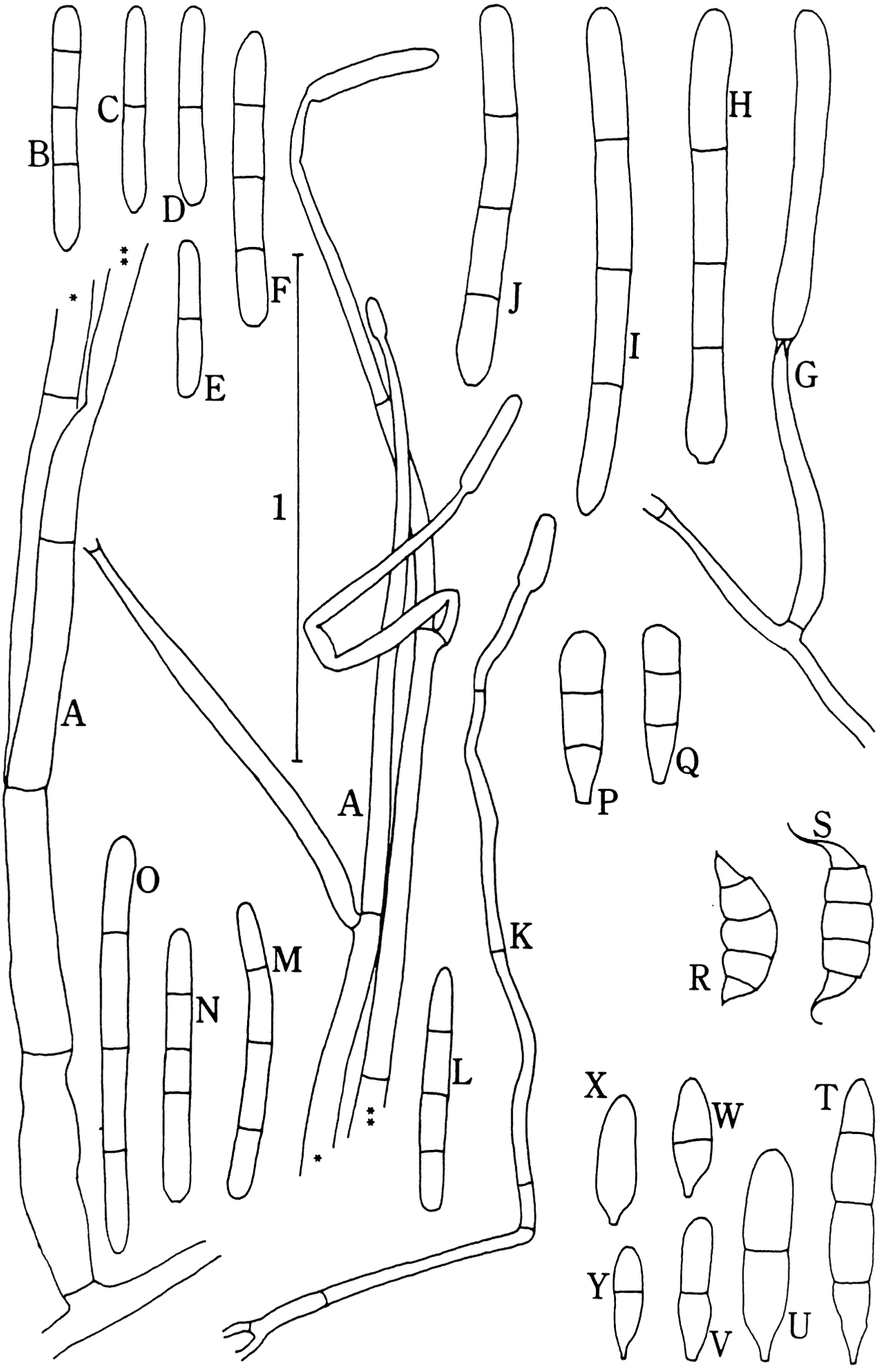
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Fig. 3.11

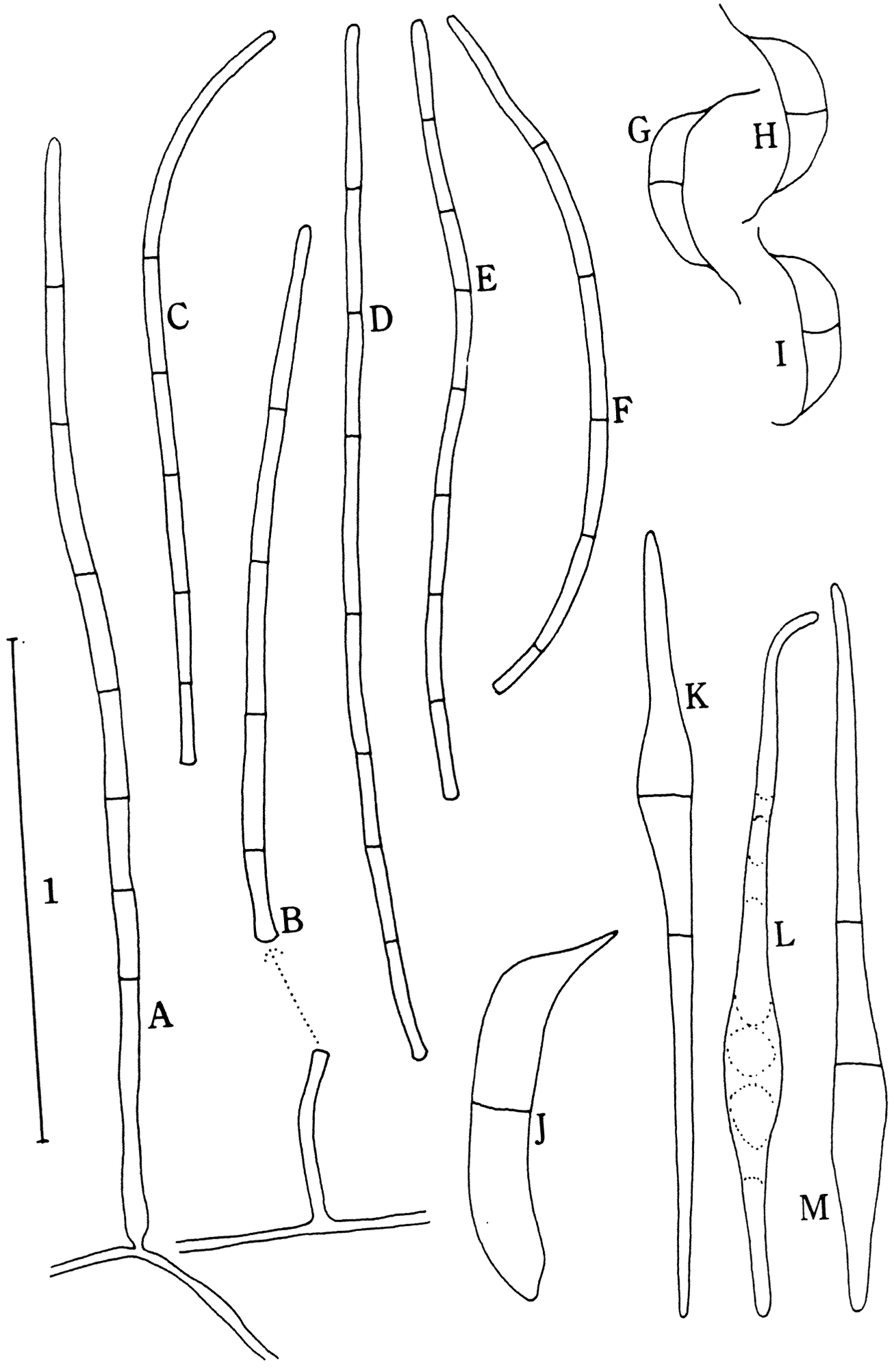
Scale 1 = 50 μm.

A -F Attached and detached conidia of Unidentified sigmoid/rod type 18, A,B,C,E from isolation agar on which beech leaves from Reid's CK were plated, D from beech leaves from Reid's Ck, F from random leaves from Hannah Ck (South Island).

G,H,I Unidentified spores from beech leaves from Hakarimata.

J Unidentified spores from rimu leaves from Hakarimata.

K,L,M Unidentified ascospores from nikau leaves from Hakarimata.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k,l,m Detached spores of Unidentified sigmoid/rod type 19, l,m from kamahi and k from beech leaves from Reid's Ck.

n,o Detached spores of Unidentified sigmoid/rod type 20 from random leaves from Nihotupu str.

p,q,r Detached spores of Unidentified sigmoid/rod type 21 from kanono leaves from Hakarimata.

s,t Detached spores of Unidentified sigmoid/rod type 22 from kanono leaves from Hakarimata.

u Detahed spore of *Tricladium* sp. from Rock Burn (South Island) stream filter.

v,z,aa Unidentified tetaradiate spores from Reid's Ck stream filter.

w *Volucrispora graminea* Ingold -like spore from random leaves from Reid's Ck.

x Unidentified tetraradiate spore from McTavish Ck (South Island) stream filter.

y Detached spore of *Tricladium* sp. from Reid's Ck stream filter.

bb *?Tricladium anomalum* Ingold spore from beech leaves from Reid's Ck.

cc Detached spore of *?Ceratosporium* sp. from beech leaves from Reid's CK.

Fig. 3.12

Scale 1 = 50 μm; scale 2 = 100 μm.

(F,G,H,I,k,l,m,n,o,s,t = scale 2, rest scale 1)

A -E Detached spores of Unidentified sigmoid/rod type 10, C,D from isolation agar following plating of kamahi leaves from Hakarimata, A from beech leaves from Reid's Ck, B,E from kamahi from Hakarimata.

F,G Detached spores of Unidentified sigmoid/rod type 11 from isolation agar on which kamahi leaves from Reid's Ck were plated.

H,I Detached conidia of Unidentified sigmoid/rod type 13 from isolation agar on which kamahi leaves from Hakarimata were plated.

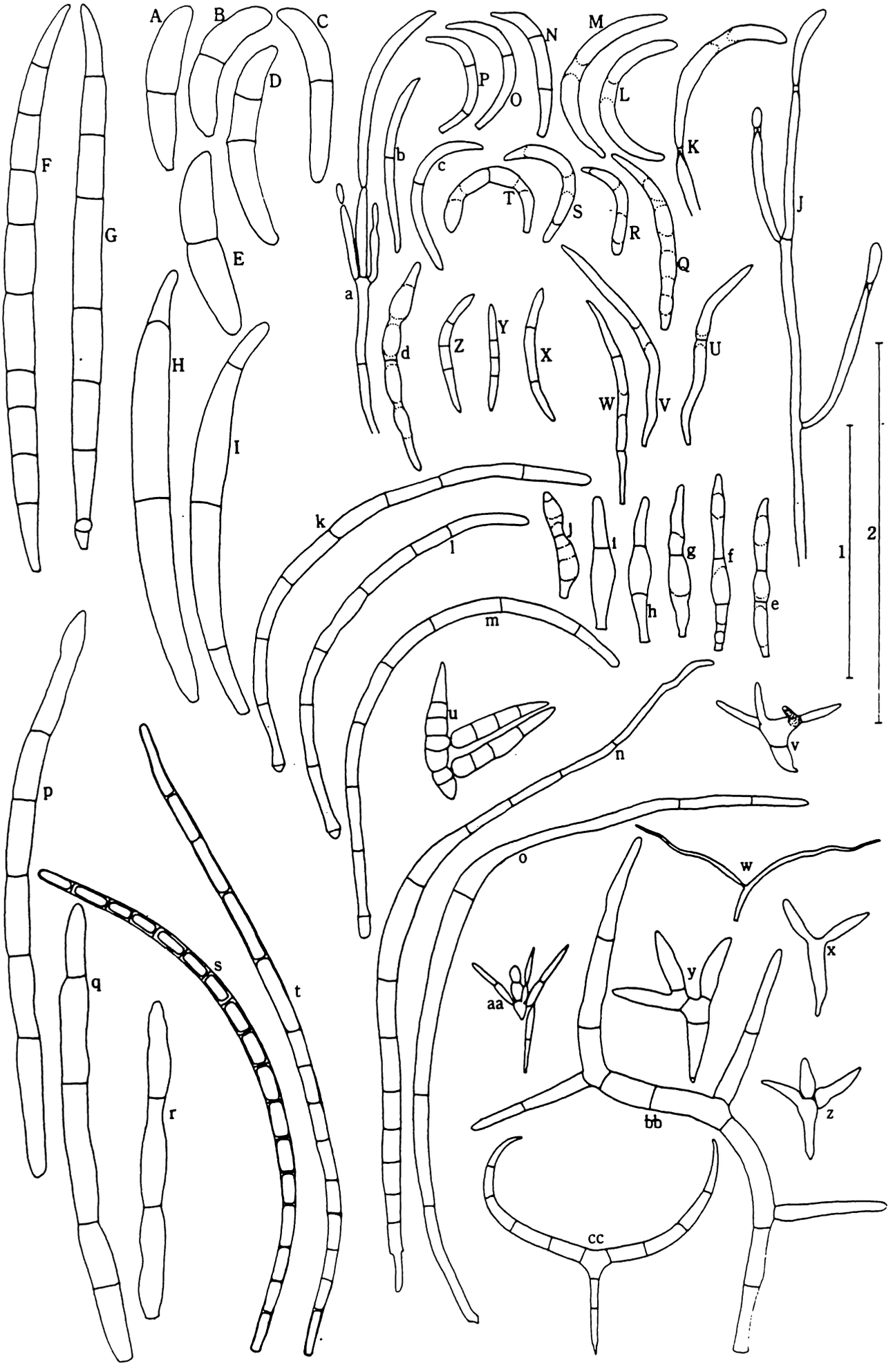
J -T Attached and detached conidia of Unidentified sigmoid/rod type 14, J -M from isolation agar following plating of kamahi leaves from Reid's Ck, N,O,P from random leaves from Reid's Ck, Q -T from random leaves from Rock Burn (South Island).

U,V,W Detached spores of Unidentified sigmoid/rod type 15 from random leaves from Reid's Ck.

X,Y,Z Detached spores of Unidentified sigmoid/rod type 17 from kamahi leaves from Reid's Ck.

a,b,c,d Attached and detached conidia of *Flagellospora penicillioides*, a,b,c from isolation agar following plating of beech leaves from Hakarimata, d from beech leaves from Hakarimata.

e -j Detached spores of Unidentified sigmoid/rod type 16, f,g from kamahi leaves from Hakarimata, e,h,i,j from beech leaves from Hakarimata.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Unidentified sigmoid/rod type 18

Fig. 3.11A -F

Conidia such as these were seen attached rarely during the plating experiment but more commonly as detached conidia only in the plating and leaf bag experiments. The conidiogenous cells seen were hyaline, short (17 -27 x 1.5 -2 μm) and simple, sessile on vegetative hyphae. The conidia are hyaline, long (49 -103 x 1.5 -2 μm) and straight, (3-) 4 -7 septate, usually tapering only slightly to each end, the attachment end flattened and anterior end rounded, the walls thin and smooth.

Sigmoidea is the most likely aquatic hyphomycete genus for this fungus. The genus has short, lateral, sympodially proliferating, holoblastic conidiogenous cells, and long unbranched conidia (Dyko and Sutton, 1978). Proliferation was not observed in the present fungus. The conidial size corresponds to that of *S. prolifera* (Petersen) Crane but this species is curved or sigmoid, and it is not clear from the description whether the conidia taper significantly or not. *S. aurantiaca* Descals and Webster is described as "straight but later falcate, sigmoid or in an extended helix" (Descals and Webster, 1982a). It is also broader than the present fungus, and tapers noticeably. *S. praelonga* Marvanová is more similar to the present fungus: "straight to slightly curved" and "slightly tapering distally" (Marvanová, 1986), but is also broader (2.5 -4 μm).

Subulispora also has short simple conidiophores and long, straight, septate conidia. According to De Hoog (1985) it keys out as *S. variabilis* De Hoog or *S. cylindrospora* P. M. Kirk. *S. variabilis* differs from the present species in having pale brown conidiophores (and conidiogenous cells) and slightly shorter, broader conidia. *S.*

cylindrospora differs in having macronematous, pigmented conidiophores and shorter conidia (Kirk, 1985).

Unidentified sigmoid/rod type 19

Fig. 3.12k, l, m.

These spores were seen in the leaf bag experiment, as detached spores only. They are reminiscent of type 18, but are slightly larger, and strongly curved. Because there is little evidence of conidiophore and conidiogenous cell characteristics a definite identification cannot be made. However, spore characteristics such as spore size, septation, shape (usually cylindrical and curved), and the conspicuous attachment scar, commonly with proliferation through it, suggest possible affinities. *Anguillospora curvula* Iqbal is the only *Anguillospora* species with conidia small enough to match the present fungus, although they are usually slightly larger, and gradually taper towards the apex. Conidia of *Pseudoanguillospora* species also are released by disarticulation at the basal septum, as the present spores appear to be. Those of *P. prolifera* Iqbal are the same width, but twice as long, and only slightly curved. *P. gracilis* Sinclair and Morgan -Jones conidia match the present spores well in length, but are narrower, less septate, and only slightly curved. *Sigmoidea prolifera* is clearly a strong possibility, although the conidia may taper, and the range of conidium widths includes conidia half the width of the present spores.

Unidentified sigmoid/rod type 11 (Fig. 3.12F, G) possibly also belonged in *Anguillospora*.

Unidentified sigmoid/rod type 20

Fig. 3.12n, o.

These conidia, seen in the stream survey in the Nihotupu str. are rather like the *Mycocentrospora* sp. depicted in Aimer and Segedin (1985b) except that they lack a basal appendage. They are thus most similar to *M. angulata* (Petersen) Iqbal, being only slightly narrower than the minimum width recorded for this species, but more strongly tapered, and the septa distributed asymmetrically.

Unidentified sigmoid/rod type 21

Fig. 3.12p, q, r.

This group comprises large, septate rod -shaped spores. They are rather variable and may not all belong to the same species.

Unidentified sigmoid/rod type 22

Fig. 3.12s, t.

These large spores had greatly thickened walls in parts, for example, the anterior half only, or only at the apex and base of the spore. They were seen only occasionally, in the leaf bag experiment.

Unidentified sigmoid/rod spores

A large number of sigmoid and rod shaped spores were seen only very rarely, and as detached spores only, in the leaf bag experiment and

stream survey. These were counted simply as Unidentified sigmoid/rod spores. Fig. 3.11J (possibly a *Rhynchosporium* sp.) comprised most of the unidentified sigmoids on stream filters from Reid's Ck and Hakarimata.

Unidentified tetraradiate spores.

These spores were similarly seen only once, or very rarely. Some are shown in Fig. 3.12u -z and aa, bb, cc, together with possible identifications.

Unidentified spores.

On occasion other amerospore or didymospore types were seen. On some leaf types, notably nikau, several types of ascospores, both oval and filiform, were seen in significant numbers. They were not identified, but a most distinctive spindle -shaped type is depicted in Fig. 3.11K, L, M.

CHAPTER FOUR

RESULTS 2: MEASURMENT OF MYCELIAL LENGTHS OF AQUATIC HYPHOMYCETES FROM SPORE PRODUCTION.

Introduction.

The aim of the present study was to examine the role of aquatic hyphomycetes in leaf decay and their importance in different seasons and stream types. To do this a method of measuring aquatic hyphomycete mycelial content from spore production was developed. This chapter gives the results of the experiments developing the sporulation technique. The leaf bag experiment and the stream survey, which used this technique, are presented in chapter 5. The results of experiments using different methods (selective inhibition of respiration and particle plating) to measure the relative importance of aquatic hyphomycetes compared with other microbial groups in litter in streams, are given in Chapter 6.

Aquatic hyphomycetes generally produce abundant, readily identifiable spores. For this reason studies of aquatic hyphomycetes on leaves in streams have quite commonly used the number of spores produced during a brief incubation period as a measure of relative abundance, for comparative purposes. However, the technique has been criticised for selecting for prolifically sporulating species, and for probably being influenced by the substrate type and stream factors (Chamier et al, 1984; Bärlocher, 1982a). In the present work it was decided that it might be possible to quantify and compensate for these factors, and to use spore production as a measure of vegetative biomass. There has been

little previous work on the sporulation of aquatic hyphomycetes, of the relationship between spore production and vegetative mycelium, including what factors induce sporulation. In the present work, then, some experiments were undertaken to test the factors inducing sporulation in aquatic hyphomycetes. Experiments were also carried out to calculate mycelium/spore conversion factors for individual species, to allow for differing sporulation capacities between species. Following this, some of the conditions which differed between pure culture (in vitro) and naturally occurring leaves (in vivo) were tested for their effect on the mycelium/spore conversion factors derived from pure culture.

The present chapter is divided into three sections. These are:

4.1 Examination of the factors inducing or controlling sporulation in aquatic hyphomycetes in pure culture.

4.2 Measurement of the mycelium/spore conversion factors of aquatic hyphomycetes in pure culture, and testing the effects of factors which might affect the use of the mycelium/spore figures on natural leaf material.

4.3 Development of a standard method for measuring spore production from leaves collected from streams, and the role of some of the standardised factors in inducing sporulation in vivo.

4.1 FACTORS CONTROLLING SPORULATION OF AQUATIC HYPHOMYCETES IN PURE CULTURE.

A wide variety of factors have been found to induce sporulation in fungi in general, including nutrient depletion (either C or N), light, partial desiccation, cutting the mycelium, changes in

temperature, toxins, changes in mycelial age, and osmotic shock (Smith and Berry, 1974; Cole, 1986). The typical method of inducing sporulation of aquatic hyphomycete cultures is to submerge pieces of the culture in deionised water. This method is unique among conidial fungi, most of which do not sporulate under submerged conditions. Preliminary work showed no sporulation occurred on dry agar (1.5 % malt agar) in any of the species isolated from New Zealand streams, while abundant spore production normally occurred following submersion of pieces of the agar in deionised water. This followed the same pattern as was found in overseas studies (Nilsson, 1964; Ingold, 1975; Webster and Descals, 1981).

Spore induction following submersion in deionised water could be the result of a reduction in nutrient concentration. Previous workers have found C or N limitation can induce sporulation in other fungi (Smith and Berry, 1974). The aim of the next few experiments was to test for the effect of nutrient deprivation or excess on sporulation -whether nutrient concentrations could affect sporulation in a similar fashion to that in other, terrestrial, fungi, and whether nutrient deprivation is the effect of submersion which induces sporulation. The first experiment used malt extract as the nutrient source, and thus did not separate C and N. This was done in the second, liquid culture, experiment.

4.1.1 Effect of nutrient concentration.

Sporulation might be induced either by providing low nutrient conditions, if the absolute nutrient level was the controlling

factor, or by causing a reduction in the available nutrients, if the relative levels were more important. In the first experiment the ability of low nutrient levels to induce sporulation alone, without submersion, was tested by growing two species on very weak media compared to the normal 1.5 % malt agar. The effect of a change in nutrient concentration was tested by incubating mycelium from each agar strength in nutrient solutions of the same range of strengths.

The two species (*A. filiformis* and *F. penicillioides*) were grown on 2 strengths of malt agar (1.5 % and 0.015 %) and on water agar. Radial growth rates were largely unaffected by decreased substrate concentration, or even increased slightly in the case of *A. filiformis*. However, on 0.015 % malt and water agar the density of the hyphae decreased and colonies were pale, with aerial mycelium reduced or absent compared with those on 1.5 % malt agar. Sporulation was measured following incubation of agar discs cut from the colony margins of each species in a corresponding series of solutions. The amount of mycelium present was measured prior to incubation, as in the standard method. The sporulation results are given below in Table 4.1.1a and 4.1.1b. Because the mycelial content of the agar discs was measured only at the beginning of the experiment and may have altered during the experiment, particularly in the presence of malt solution, the figures for spores/m mycelium (Table 4.1.1b) were calculated for day 3 only.

Table 4.1.1a: Spores produced from discs of *A. filiformis* and *F. penicillioides* grown on three media types (1.5 % and 0.015 % malt agar, and water agar), then incubated in the same range of solutions (1.5 % and 0.015 % and deionised water). Spore production was measured at intervals and is given as number of spores per agar disc per day (mean/standard deviation x 10^2).

A. filiformis	Spores/disc			
	Day 1	Day 3	Day 6	Day 12
agar/solution				
1.5/1.5	0	0	0	0
1.5/0.015	0	5/4	316/157	148/100
1.5/water	0	41/13	65/60	6/4
0.015/0.015	0	2/1	152/75	140/37
0.015/water	0	7/6	5/2	1/0
water/water	0	2/1	7/1	1/1
1.5/1.5	0	0	0	0
1.5/0.015	0	164/126	643/246	268/176
1.5/water	0	249/80	909/442	68/25
0.015/0.015	0	72/48	1623/556	222/26
0.015/water	0	221/59	62/11	1/0
water/water	0	89/11	54/13	1/0

Table 4.1.1b: Sporulation of *A. filiformis* and *F. penicillioides* grown on three agar types (1.5 % and 0.015 % malt agar, and water agar) and incubated for three days in the same range of solutions (1.5 % and 0.015 % malt solution, and deionised water), given as spores produced per m mycelium.

	Spores/m mycelium	
	A. filiformis	F. penicillioides
agar/solution		
1.5/1.5	0	0
1.5/0.015	4.2	360
1.5/water	38.0	530
0.015/0.015	4.2	100
0.015/water	19.0	310
water/water	0.6	180

Where similar differences occurred in both species they were considered significant even though not statistically significant in either species individually, because of the high variances encountered.

There was no sporulation on the first day (Table 4.1.1a), indicating no sporulation was occurring on agar of any nutrient strength before submersion. Thus low nutrient levels alone were not sufficient to induce sporulation. Submersion in solutions of the same concentration or lower was necessary for sporulation to occur even on low nutrient agar.

High levels of malt in the incubation water totally suppressed sporulation in both species. Low concentrations of malt resulted only in slightly lower sporulation during the first three days

compared to the deionised water treatments. Following this higher spore production occurred than was achieved at any time during incubation in deionised water (Table 4.1.1a). The increase in spore production over that obtained in deionised water could result from an increase in vegetative mycelium in the first few days, or to less mycelial death through starvation prior to sporulation, or to to external nutrients being used to support sporulation directly. It is not possible to tell whether these high levels of sporulation occurred only after the malt was used up, or whether sporulation was simply slower to attain maximum levels in the presence of a nutrient source.

A change in nutrient concentration was not necessary for sporulation to occur (0.015/0.015 sporulated very abundantly), but initial spore production (Day 3) followed the pattern of differences in malt concentration between agar and solution. Thus 1.5/water and 0.015/water showed markedly higher levels of sporulation than 1.5/0.015 and 0.015/0.015 respectively (Table 4.1.1b). This might represent synchronous stimulation of a greater part of the mycelium to sporulate immediately due to more rapid nutrient losses from leaching, a more complete absence of external nutrient sources to maintain vegetative growth.

These results suggest that in-vitro sporulation at least only occurs when the fungus is submerged and nutritionally deprived. Above a certain concentration nutrients are used for vegetative growth (seen in abundance in the 1.5/1.5 treatment) at the expense of sporulation. Below this point the nutrients present may be used in spore production at least in part.

In the second experiment the effect of nutrient concentration was examined more closely by separating the effect of the carbon source from the effect of other nutrients in a defined medium.

This experiment was repeated once, *L. cymbiformis* being the test species on both occasions. In the first trial 15 mycelial inocula were prepared (2.1.1) and divided among the following five treatments:

1) transferred to fresh high glucose (10 g/l) + full strength mineral nutrient solution;

2) transferred to low glucose (1 g/l) + full strength mineral nutrient solution;

3) transferred to high glucose + low mineral nutrient solution (10 %);

4) transferred to low glucose + low mineral nutrient solution;

5) left in original (week old) growth medium to which fresh high glucose + full strength mineral nutrient medium was added (50:50 by volume), so that the mycelium was not taken out of the liquid or disrupted at all.

Each 125 ml flask contained 50 ml medium, with the initial inoculum weighing 0.012g (dry weight). Sporulation was measured every two days during the 8 day incubation period, by filtering a small quantity of medium, and the mycelial content of the flasks was determined by filtering, drying, and weighing at the end of the experiment. The results are given in Table 4.1.1c.

Table 4.1.1c: Spore production and dry weight production of *L. cymbiformis* in submerged culture in four different combinations of fresh high and low concentrations of glucose and of mineral nutrients, and in a high glucose + mineral medium only half of which was fresh. The results are given as spores/flask (mean/S.D.), measured every two days, followed by g (D.W.) of mycelium produced per flask during the 8 day incubation, then spores/g mycelium (using the Day 8 spore production figures).

	Medium Type				
	50:50 unchanged	High C /high M.	Low C /high M	High C /low M.	Low C /low M.
Spores/flask					
Day 2:	37/12	0	3/6	7/6	63/93
Day 4:	4/7	8/14	6313/7369	171/166	150/142
Day 6:	0	146/252	242390/70425	4424/5726	11584/7304
Day 8:	34/47	653/543	217174/62691	6946/1807	29817/20509
D.W.:	0.142g	0.174g	0.025g	0.188g	0.029g
Spores/g (Day 8):		3,764	8,550,158	36,849	1,021,130

Sporulation/g mycelium was over 1000 times higher in the low C/high mineral solution than in the full nutrient solution (high C/high mineral solution), indicating that availability of C is the more important factor in inducing or inhibiting sporulation. However, the high C/low mineral treatment sporulated 10 times more than the high C/high mineral treatment, indicating that the level of at least one nutrient other than C is important to the induction of sporulation, low levels stimulating sporulation to some extent when levels of C are high. Where low levels of C occurred the

sporulation induced was enhanced by higher levels of other nutrients (low C/high mineral solution compared with low C/low mineral solution).

Levels of sporulation in the full nutrient "50:50 unchanged" treatment were low and variable throughout the experiment. In comparison, in the high C/high mineral treatment sporulation was initially low but showed a clear pattern of increasing numbers in the last few days. The difference between the two may relate to the presence of some sort of sporulation-inhibiting staling products in the "50:50 unchanged" flasks. Alternatively, if the stimulating factor in the high C/high mineral treatment is nutrient limitation, then possibly the slightly slower growth rate in the "unchanged" flasks did not deplete the medium to the same extent during the length of time available.

The increasing spore numbers with time seen in all except the "50:50 unchanged" flasks may be attributable to depletion of one or more critical nutrients, the response to nutrient limitation being reduction in vegetative growth rate and initiation of spore production. In the second trial of this experiment mycelial dry weights were measured several times during the experiment in order to observe the relationship between the cessation of vegetative growth and the commencement of sporulation.

L. cymbiformis was grown in full strength nutrient solution for a week prior to transferring to fresh nutrient solutions, as in the first experiment. Because of the increased size of the experiment only three treatments were used (high C/high mineral solution, high C/low mineral solution and low C/high mineral solution). Extra flasks of each were set up to allow the (unreplicated) determination of mycelial lengths during the experiment (by filtering and weighing as above). The results are given below in Tables 4.1.1d and 4.1.1e.

Table 4.1.1d: Spore production of *L. cymbiformis* in submerged culture in three different combinations of high and low concentrations of glucose and of mineral nutrient solution. The results are given as spore production/flask (mean/standard deviation).

	Medium Type		
	High C/high M.	High C/low M.	Low C/low M.
Day 0:	sporulation not measured		
Day 2:	0	0	0
Day 3:	0	0	0
Day 5:	0	0	355/390
Day 6:	0	0	480/628
Day 8:	0	0	3989/3847
Day 9:	0	0	15330/11761
Day 13:	0	20/30	40296/27068
Day 19:	3801/4799	14820/24688	34748/6382

Table 4.1.1e: Dry weight production of *L. cymbiformis* in submerged culture in three different combinations of high and low concentrations of glucose and of mineral nutrient solution. The results are given as g (D.W.) of mycelium per flask.

	Medium Type		
	High C/high M.	High C/low M.	Low C/high M.
Day 0:	0.043	0.043	0.043
Day 3:	0.239	0.126	0.111
Day 6:	0.361	0.309	0.099
Day 9:	0.603	0.326	0.098

The same pattern of increasing sporulation appeared in the low C/high mineral nutrient medium as was seen in the first trial of this experiment. The level of maximum sporulation per g mycelium was somewhat lower: c. 5×10^5 compared with 8×10^6. This difference may be attributable to the slower spore induction occurring in the second experiment, so that sporulation was spread over a longer period.

The relationship between growth rate (Table 4.1.1e) and sporulation (Table 4.1.1d) is only discernible for the low C/high mineral solution treatment, because sporulation did not start until very late in the other treatments (after the weight loss replicates had been used up), due to the lower mycelium:medium volume compared with the first trial. In the low C/high mineral solution treatment vegetative biomass had reached its maximum by Day 3, while sporulation became apparent two days later. Thus sporulation commenced after vegetative growth had ceased.

4.1.2 Effect of sporulation on mycelium.

In the nutritional experiments given above sporulation appeared to occur in conditions unsuitable for the support of vegetative growth, that is, after nutrient depletion. This being the case, the very considerable energy and materials required for the production of spores are presumably obtained by recycling vegetative biomass. This was examined in experiments using agar discs, measuring the stainable biomass (mycelium with cell contents) before and after sporulation.

Five species, grown on a variety of media were tested, *A. pulchella* (as young and old mycelium), *L. ovalis* sp. ined., and *L. cymbiformis* on malt agar; and *C. aquatica*, *L. cymbiformis*, *L. ovalis*

sp. ined., and *T. chaetocladium* on leaf agar. Replicate sets of discs were cut from each, to measure mycelial content from some immediately (using trypan blue in water), and from others following a two week incubation in deionised water to induce sporulation, as in the standard method. The results are given in Table 4.1.2 .

Table 4.1.2 : Percentage of total hyphae stained with trypan blue before and after sporulation. The four species were grown on malt agar (discs cut from older or younger areas as indicated), or on leaf agars of two concentrations, with or without added mineral nutrients (N). "NM" = not measured.

Species	Medium	Stained Mycelium	
		before	after
A. pulchella	malt -young	58 %	NM
	malt -old	54 %	26 %
C. aquatica	leaf -10 g/l + N	80 %	23 %
	leaf -10 g/	87 %	NM
	leaf -1 g/l + N	80 %	NM
L. cymbiformis	malt -young	NM	59 %
	malt -young	NM	22 %
	malt -young	96 %	20 %
	malt -young	84 %	NM
	leaf -10 g/l + N	74 %	16 %
	leaf -10 g/l + N	80 %	NM
	leaf -1 g/l + N	62 %	NM

Table 4.1.2b cont.

Species	Medium	Stained Mycelium	
		before	after
L. ovalis sp. ined.	malt -young	50 %	NM
	malt -young	48 %	NM
	leaf -10 g/l + N	84 %	NM
	leaf -10 g/l + N	85 %	NM
	leaf -10 g/l	85 %	NM
	leaf -1 g/l + N	78 %	NM
T. chaetocladium	leaf -10 g/l + N	47 %	20 %
	leaf -10 g/l + N	33 %	NM
	leaf -10 g/l	44 %	NM
	leaf -1 g/l + N	62 %	NM

With the exception of one *L. cymbiformis* measurement (which may have been an overestimate), only about 20 % of the total mycelium was stainable following sporulation in all species tested. The percentage of total mycelium stainable prior to induction of sporulation varied somewhat, but was invariably considerably higher. Thus when stained mycelium was measured both before and after sporulation the proportion of total mycelium stainable was reduced by at least half, and considerably more when the stained proportion was much greater than 50 % initially.

It is suggested that sporulation under the standard conditions used in the present work "uses up" vegetative mycelium in the production of spores, that sporulation may be, in effect, the conversion of a proportion of vegetative mycelium into spores. If this is the case, then under standard conditions, a set proportion of the mycelial biomass of a particular species will be converted into a set number of spores, and the amount of mycelium present could therefore be calculated from the number of spores produced by a fungus.

4.2 PRODUCTION OF MYCELIUM/SPORE CONVERSION FACTORS.

The purpose of the experimental work detailed in this section was to measure the relationship between vegetative mycelium and the number of spores produced in pure culture, to obtain mycelium/spore conversion factors. Because measuring total number of spores produced would be time-consuming and difficult, the maximum daily rate of spore production was used instead. Measured under constant conditions this ought to be proportional to total number, within each species, as spore production increased to a peak then decreased again in a repeatable parabolic fashion. The reliability of these assumptions, and therefore the usefulness of the calculated mycelium/spore conversion factors in natural field -collected substrates was tested by considering the effect on them of the conditions which could have differed in naturally collected substrates compared with pure cultures on malt agar. These were: C source and concentration during growth, concentration of other nutrients during growth, the presence of naturally occurring competitors, previous low oxygen conditions, and the shape of the substrate. The mycelium/spore values were also tested against mycelium/respiration values, which were measured from the same agar cultures. Both respiration and sporulation could then be used to calculate the mycelial content of sterile leaves inoculated with aquatic hyphomycete species, thus testing each other.

4.2.1 Measurement of mycelium:spore ratio on malt agar.

In a series of experiments 13 aquatic hyphomycete species were grown on 1.5 % malt agar, then discs were submerged in deionised

water and sporulation followed over a two week period by filtering every few days, as in the standard method (2.1.4). Most species were shaken during incubation, as in the standard method, while others were incubated in still conditions in addition to shaken incubation. The agar discs used for submersion were cut from old parts of the colonies and from the young, growing margins. Total mycelial measurements (stained and unstained) were made at the beginning of submersion. Thus observations of the effect of mycelial age and of agitation, on sporulation were possible from this experiment, in addition to the main purpose of the work, that of measuring the mycelium/spore relationship. The figures for mycelium/spore values obtained are given in Table 4.2.1.

Table 4.2.1: Total amount of mycelium (mm) required to give rise to one spore at maximum rates of spore production in thirteen species all grown on 1.5 % malt agar. Mycelium was obtained from older or younger parts of the colonies, and was incubated in either shaken or still conditions.

Species	Treatment	Mycelium per spore
A. pulchella	young, shake	520
	old, shake	100
A. filiformis	young, still	6.3
	old, still	11
	young, shake	65
A.longissima	young, shake	100
	old, shake	370
A. moniliformis	young, still	1.4
A. tetracladia	young, still	40
	old, still	5.1
	young, shake	1.9

Table 4.2.1 cont.

Species	Treatment	Mycelium per spore
C. aquatica	young, still	40
	old, still	8.2
	young, shake	46
	old, shake	24
F. annelidica	young, still	5.5
	old, still	2.5
F. penicillioides	young, still	0.46
	old, still	0.96
	young, shake	0.23
	old, shake	0.25
L. curvula	young, shake	1.1
	young, shake	4.0
L. cymbiformis	young, shake	6.1
L. ovalis sp. ined.	young, shake	6.0
	old, shake	1.0
	young, shake	7.5
	old, shake	0.8
T. chaetocladium	young, still	41
	old, still	60
	young, shake	45
	old, shake	880
V. elodeae	young, still	6.6
	old, still	4.4
	young, shake	11

The variation in mycelium/spore value within a species was quite high, limiting the reliability of the values obtained as sporulation to mycelium conversion factors for species in which the determination was not repeated. This high variability possibly also

obscures the effects of the different treatments. In future work this might be reduced by measuring sporulation every day, to ensure the maximum rate is obtained.

The effect of age was also obscured by the difference in growth rate between species. This resulted in the agar discs containing mycelium of widely varying actual ages, and therefore only the relative differences between "young" and "old" discs being comparable between species. In four species smaller mycelium/spore values were obtained from young samples, but in 5 species the reverse pattern appeared. However, the differences in mycelium/spore values between older and younger parts of colonies were generally of a similar magnitude to the differences seen between repeat experiments using the same age mycelium, suggesting the differences in age were not important.

Although more species showed slightly higher maximum sporulation in still incubation than in shaken (lower mycelium/spore figure), the difference was marked only in the case of *A. filiformis*. As the experiment was not repeated, this may have been a chance result. The reverse pattern appeared in *F. penicillioides*, with somewhat higher sporulation occurring when the submerged cultures were shaken.

Similarly, it was noticed there was no consistent difference in the time taken for spore numbers to increase or to reach a maximum in most species. Sporulation increased slightly faster in shaken cultures of *A. filiformis* and *F. penicillioides*, but only by one day.

Thus shaking the incubating cultures did not appear to have a significant effect on sporulation.

4.2.2 Effect of C substrate type on the mycelium:spore ratio.

In the previous experiment mycelium/spore values were measured for species on a stock medium (malt agar). This provides a simple carbon source (maltose) which is unlikely to be significant in the normal substrate of aquatic hyphomycetes, that is, leaves in streams. The applicability of these values to fungi growing on different carbon sources, particularly more natural and complex carbohydrates, was tested in the present experiment.

Three species (*L. cymbiformis*, *T. chaetocladium*, and *L. ovalis* sp. ined.) were grown on four increasingly complex carbon sources which occur naturally in leaves: glucose, starch, pectin, and cellulose, each made up into agar (2.1.1). Discs from the colony margins were submerged in deionised water to stimulate sporulation, and the total mycelial content of further replicate discs measured, using standard methods. The experiment was repeated once.

Growth and sporulation of all three species occurred on all substrate types. The density of mycelium, measured from close to the colony margin, was closely similar on all media, only that on cellulose agar being slightly less dense. No direct test was made of the extent to which the carbon substrates were actually being utilised, however. In the first trial there was no difference between the same species on different carbon sources in the time taken to reach maximum sporulation following submersion, but there was considerable variation between the species. In the second trial all three species reached a maximum after 2 -3 days on glucose, starch, and pectin agar, but not until Day 6 on cellulose agar. Thus it is possible that cellulose was a poorer substrate for the aquatic hyphomycete species tested than any of the other types used, that is, supported less rapid vegetative growth, if not sporulation.

Mycelium/spore values were calculated from the maximum sporulation rate obtained over the incubation period. The results for both trials are given in Table 4.2.2.

Table 4.2.2: Mycelium/spore conversion factors calculated on two separate occasions (trial 1 and 2) for *L. cymbiformis*, *T. chaetocladium*, and *L. ovalis* sp. ined. grown on glucose, starch, pectin, and cellulose agar. Results are given as mm mycelium per spore.

Medium	Mycelium per spore values.					
	L. cymbiformis		T. chaetocladium		L. ovalis	
	trial 1	trial 2	trial 1	trial 2	trial 1	trial 2
Glucose	3.2	3.6	41.0	4.8	3.7	2.0
Starch	3.6	1.3	41.0	32.0	4.0	1.6
Pectin	4.7	2.8	19.0	27.0	0.8	13.0
Cellulose	6.7	8.1	16.0	47.0	4.4	4.1

There was little difference between mycelium/spore values on the different carbon sources, including cellulose. Only *L. cymbiformis* gave slightly higher mycelium/spore values on cellulose agar in both trials, but the difference was not great compared to the variation within other species and between species. This indicates that the mycelium/spore conversion factors calculated for species growing on malt agar are applicable on different substrate types, including those occurring naturally in streams. This was

further tested in the next experiment (4.2.3), using ground leaf agar as the substrate.

A minor aim of the present experiment was to look for evidence of sporulation on dry agar, i.e. without submerging, on the different media types. The plates from which discs were taken for submersion in the second trial were washed and the water filtered and examined for spores. No spores were obtained from the glucose and cellulose plates. Some spores of each species were found on the pectin plates, although mostly rather small, abnormally shaped spores, and only 48 -211 spores from plates each with two whole colonies of 2 -3 cm diameter. Very few spores were seen on the starch plates of *L. cymbiformis* and *L. ovalis* sp. ined. (6 spores per plate), but slightly larger numbers of *T. chaetocladium* (268 spores from the whole plate). Thus there was little or no sporulation on dry agar on these media, as was found on malt agar plates used in the earlier spore induction work. What sporulation there was may have varied between agar types, being greater on pectin and starch, although the observations would need to be repeated in order to rule out differences in condensation between plates, for instance.

4.2.3 Effect of natural substrates and inorganic nutrient concentrations on the mycelium:spore ratio.

The media used in experiment 4.2.2 (above) to measure the mycelium/spore conversion factors were still very different biochemically from naturally occurring leaves, being pure and artificial. The effect of the wider range of nutritional sources in leaves, or the presence of inhibitory compounds (tannins etc) might stimulate or reduce spore production. In this experiment agar containing leaf powder represented a more natural substrate, but still allowed the easy measurement of mycelial length.

Leached, macerated leaves were used as a C source, at 1 and 10 g/l. It is not known whether additional mineral nutrients may be obtained from stream water, so to allow for this a mineral nutrient source was added to some plates but not others. Because the macerated leaves tended to sink slightly before the agar set, and most mycelial growth occurs in the upper part of the agar, the agar in some plates (10g/l + mineral nutrients) was cut out and inverted before inoculation.

Four species were used, although contamination or other errors reduced this to three for some treatments. Agar discs were cut from the colony margins, submerged, and sporulation followed, as in the standard method.

There was no significant difference between the treatments in the time taken by *L. cymbiformis* to reach maximum sporulation, which appeared in a similar length of time to that normally taken on malt agar (4 -5 days). The remaining three species varied more between treatments (3 -6 days), and appeared to be slower to reach a maximum on the 1 g/l plates (6 -12 days), although the less frequent sampling over this later period may have distorted the results. Thus there was no clear connection between substrate or nutrient

concentration and the time taken to induce maximum sporulation even where the C source was insoluble and so not diluted by submerging.

The mycelial contents of separate, replicate discs were measured at the time of submersion. In this experiment stained (trypan blue) and unstained hyphae were measured separately. Stained mycelium comprised 62 -80 % of the total mycelium of *L. cymbiformis*, 33 -62 % of that of *T. chaetocladium*, 78 -85 % of that of *L. ovalis* sp. ined., and 80 -87 % of that of *C. aquatica*. Total mycelium/spore and stained mycelium/spore values were calculated separately from the maximum spore production recorded. These are given below, in Table 4.2.3.

Table 4.2.3: Mycelium/spore conversion factors calculated for *L. cymbiformis*, *T. chaetocladium*, *L. ovalis* sp. ined., and *C. aquatica* grown on four agar types (10 g/l leaf agar made up with 10 % mineral nutrient solution and inverted, 10 g/l leaf agar with mineral nutrient solution, 10 g/l leaf agar, 1 g/l leaf agar with mineral nutrient solution. The conversion factors are given as mm total mycelium per spore, followed by mm trypan blue stained mycelium per spore.

Medium.	Mycelium per spore.							
	L. cymb.		T. chaet.		L. ovalis		C. aquat.	
	total	live	total	live	total	live	total	live
10 g/l + minerals (inverted)	3.3	2.1	31	14	2.4	2.0	17	14
10 g/l + minerals	7.5	6.0	120	39	1.7	1.5	-	-
10 g/l - minerals	-	-	34	15	2.9	2.4	35	30
1 g/l + minerals	1.2	7.1	190	120	8.3	6.4	50	40

There was no difference between treatments which was consistent in all species. Although the mycelium/spore values of the different treatments varied by up to four times within a species, this amount of variation was seen in other experiments between replicates of the same treatment. Thus this experiment confirms both the applicability of mycelium/spore conversion factors on natural substrates and the level of variation occurring between independent measures of both spore production and mycelial content.

4.2.4 Effect of natural microbial competitors on the mycelium:spore ratio.

The mycelium/spore conversion factors have so far been measured using pure cultures of aquatic hyphomycetes. The presence of competing microorganisms may affect sporulation. For example, Hawker (1959) found that the fruiting of *Melanospora destruens* (Shear) Hawker in culture could be stimulated or inhibited by "competing fungi", depending on the order of inoculation and relative growth rates. She suggested the important factors could be: reduction of food supply, production of staling substances, or production of substances favouring fruiting, and that the interactions between these could explain both stimulatory and inhibitory effects. The effect of competing fungi on the mycelium/spore values was more difficult to test for than bacteria, and was not done in the present work, as the presence of the hyphae of another species would prevent the measurement of the aquatic hyphomycete mycelium. In this experiment then the effect of natural bacterial contaminants on the relationship between vegetative mycelium and sporulation of three aquatic hyphomycete species was examined.

The test species were *L. ovalis* sp. ined., *L. cymbiformis*, and *T. chaetocladium*. Stream water was filtered through a 0.8 μm Millipore filter to remove fungi but leave at least some of the naturally occurring bacteria likely to be present in leaves from streams. Lundgren (1984) found that 50 % of the bacteria present in soils occurred in the 0.5 x 0.5 -1.0 μm size category. However, Benner et al (1986) used a 1.0 μm filter to remove fungi, and found up to 98 % of the bacteria were removed also. For this reason two treatments using the filter fractionated water were devised. In the first, 3 ml of water were inoculated onto the plates four days prior to the measurement of sporulation to allow bacterial numbers to increase and the relationship between the fungi and bacteria to equilibrate. To measure sporulation discs were cut from these plates and submerged in filter fractionated stream water. In the second treatment discs from uninoculated plates were submerged directly in filter fractionated stream water. Discs from further uninoculated plates were submerged in sterile deionised water. Sporulation was measured by the standard method in all three treatments. Mycelial contents were measured at the time of submersion.

The mycelium of one species (*L. ovalis* sp. ined.) was separated into stained and unstained hyphae to test whether the preinoculation treatment had affected the proportion of the total mycelium that was alive/had contents. There was no difference between preinoculated and sterile mycelium (both c. 50 % stained), so the results for all treatments were calculated, as in previous experiments, as total (stained and unstained) mycelium/spore from the maximum daily sporulation rate recorded during 12 days incubation (Table 4.2.4).

Table 4.2.4: Mycelium\spore conversion factors calculated for *L. cymbiformis*, *T. chaetocladium*, and *L. ovalis* sp. ined. following incubation of sterile cultures, or those preinoculated with filter fractionated stream water four days previously, in sterile water or in filter fractionated stream water. Results are given as mm total mycelium per spore.

Treatment	Species		
	L. cymbiformis	T. chaetocladium	L. ovalis
Sterile water	2.0	12	2.2
Stream water	1.8	14	3.3
Preinoculated	2.0	15	3.2

The presence of bacterial competitors clearly had no effect on the relationship between mycelium and sporulation, that is, the mycelium/spore conversion factors, for any of the species tested.

4.2.5 Effect of prior oxygen concentration on the mycelium:spore ratio.

Leaves collected from streams may have been subjected to different oxygen regimes prior to collection, which may affect the assessment of the aquatic hyphomycete mycelial content by altering the relationship between mycelium and sporulation. This effect was tested for here. In the first trial, plates with well grown colonies of the test species were gassed with a mixture of 3 % O_2, 4 % CO_2, and 93 % N_2. The plates were sealed with plastic tape and stored in a desiccator of pure N_2 for 12 days. When sporulation was assayed the O_2 content of the plates was only 1 -2 %, indicating that no diffusion of O_2 had occurred over the 12 days. Discs were

cut from the growing margins of the plates, submerged in deionised water, and spore production followed, as in the standard method. Stained hyphae were separated from the total hyphal length when it was measured prior to submersion, to check for any differences caused by the low O_2. The percentages of total mycelium which stained with trypan blue were:

96 % (control *L. cymbiformis*),

84 % (low O_2 *L. cymbiformis*),

88 % (control *T. chaetocladium*),

70 % (low O_2 *T. chaetocladium*).

Thus there was only a very slight reduction in "live" (stainable) mycelium from the controls to the low O_2 plates, and this was not statistically significant. The total mycelium/spore values were calculated from the maximum sporulation rates obtained and are given in Table 4.2.5.

Table 4.2.5: Mycelium per spore conversion factors calculated for *L. cymbiformis* and *T. chaetocladium* on malt agar with or without prior incubation in a low oxygen (3 %) atmosphere (second experiment). Results are given as mm mycelium per spore.

Treatment	Mycelium per spore	
	L. cymbiformis	T. chaetocladium
Low O_2	1.3	24
Control	2.2	19

There was no consistent or marked change in the sporulation capacity of the mycelium following low O_2 incubation. Thus the oxygenation history of a fungus does not materially affect the mycelium/spore relationship.

4.2.6 Measurement of the mycelium:respiration and respiration:spore relationship of pure cultures on malt agar.

Previous experiments (4.2.2 -4.2.5) have tested the effect of some of the conditions which could differ between pure cultures on malt agar and naturally occurring leaves in streams; that is, factors which might prevent the application of the mycelium/spore conversion factors, obtained in pure culture, to sporulation from leaves collected from streams. As a further test the mycelial content of aquatic hyphomycetes grown on sterile but otherwise natural, entire, leaves could be measured from the spore production, and checked against that calculated from the respiration rate, or more simply, by comparing the vegetative respiration/maximum spore production ratio of the same species grown on agar and on the sterile leaves (4.2.8). In order to do this, it was necessary first to measure the respiration/spore relationships of pure cultures on agar.

The respiration/spore values were measured on malt agar of three isolates of *L. cymbiformis*. Respiration per disc was measured from replicate discs extra to those submerged to assess spore production and used to measure mycelial content. Discs cut from the colony margins were incubated in fully oxygenated complete nutrient solution (10 g/l glucose + mineral nutrients) in McCartney bottles, and the rate of oxygen utilisation measured as in the standard methods for the selective inhibition of respiration experiments, omitting the antibiotics (2.1.6). The relationships between respiration and total mycelial lengths were as follows:

L. cymbiformis 4.5×10^7 mm mycelium/mg O_2/hr

3.6×10^7 mm/mg O_2/hr

3.8×10^7 mm/mg O_2/hr

From these figures, and the spore production rates, an average

figure for vegetative respiration/maximum spore production for *L. cymbiformis* can be calculated:

$$4.6 \times 10^{-7} \text{ mg } O_2\text{/hr/spore.day}$$

This figure is probably a tenfold overestimate, due to the maximum sporulation figures obtained in this experiment being ten times less than for all other experiments, probably because sporulation was not measured frequently enough to sample the true maximum. Thus only the mycelium/respiration figures should be used from this section.

The respiration rate of a known quantity of mycelium was also calculated in the experiment to determine the effect of low oxygen concentrations on sporulation (4.2.5). Discs cut from the growing margins of both control and low oxygen plates, as for sporulation and mycelial measurements, were incubated in fully oxygenated complete nutrient solution in McCartney bottles, and the rate of oxygen utilisation measured. For the purpose of converting respiration rates into mycelial measurements the total mycelium/respiration values obtained for the controls and low 02 plates were averaged:

L. cymbiformis 1.0×10^{8} mm/mg O_2/hr

T. chaetocladium 1.7×10^{8} mm/mg O_2/hr.

The vegetative respiration/maximum spore production ratios were calculated from the maximum spore production rates given earlier (4.2.5). Like the mycelium/respiration values, and the mycelium/spore values calculated earlier, there was no difference between oxygen treatments, allowing the two treatments to be averaged:

L. cymbiformis 1.7×10^{-8} mg O_2/hr/spore

T. chaetocladium 1.3×10^{-7} mg O_2/hr/spore.

4.2.7 Test of sporulation and respiration as measures of mycelial content in sterile leaves.

As mentioned above, measurements of mycelium/spore values had to be made from cultures on agar. Agar discs are artificial in terms of their structure as well as biochemistry (previously tested, 4.2.3), for example the surface area:volume ratio differed, and they lacked potential structural barriers like the cuticle. This experiment tested sporulation as a measure of mycelial content in natural sustrates against respiration as a parallel measure. It also assessed the potential for weight loss from the action of aquatic hyphomycetes alone.

Kamahi, silver beech, and ponga leaves were sterilised (2.1.2) and weighed amounts placed in 150 ml of 10 % mineral nutrient solution in 250 ml flasks, about 0.3 g dry weight of leaf material per flask. Spore solutions from cultures of *L. cymbiformis* and *T. chaetocladium* were used to inoculate the leaves: *L. cymbiformis* on all three leaf types, and *T. chaetocladium* on kamahi and beech only. Three replicates of each leaf type/fungus combination, together with three uninoculated control replicates of each leaf type were set up. The incubation fluid was replaced every week during the eleven week incubation period. At the end of this period weight loss, respiration, and sporulation were measured. The method used to measure respiration follows that of the selective inhibition of respiration experiments (2.1.5), that is the leaves were chopped up, and incubated in a glucose + mineral nutrient solution in sealed McCartney bottles to enable the change in oxygen concentration with respiration to be measured. The leaves for the sporulation measure were washed, cut up, and submerged in deionised water, in the standard method. Maximum sporulation occurred on Day 4 in all cases

except *L. cymbiformis* on beech, when it occurred on day 2.

The figures obtained for weight loss, maximum sporulation, and respiration are given below in Table 4.2.7.

Table 4.2.7: Weight loss after 77 days from sterile leaves inoculated with *L. cymbiformis* or *T. chaetocladium*, and from uninoculated control leaves, together with maximum sporulation, and respiration rates recorded from the leaves. Weight loss is given as %, sporulation as x 10^5 spores/g/day, and respiration as mg O_2/g/hour (mean/standard deviation where available).

Leaf/fungus	weight loss	sporulation	respiration
kamahi			
control	6/3	0	0
L. cymbiformis	41/4	200/48	0.21
T. chaetocladium	40/1	9/3	0.26
beech			
control	1/1	0	0
L. cymbiformis	27/6	130/78	0.21
T. chaetocladium	20/3	1/0	0.29
ponga			
control	14/2	0	0
L. cymbiformis	19/6	41/26	0.14

There was a close correspondence between spore production and weight loss in both species. This in itself was an indication that sporulation was a good measure of mycelial content. The relationship between respiration and sporulation and between respiration and weight loss was less clear, possibly because the

respiration technique was not sensitive enough for such small samples. The respiration/spore figures are:

L. cymbiformis

kamahi 1.1×10^{-8} mg O_2/hr/spore

beech 1.7×10^{-8} mg O_2/hr/spore

ponga 3.6×10^{-8} mg O_2/hr/spore

average = 2.1×10^{-8} mg O_2/hr/spore

T. chaetocladium

kamahi 2.7×10^{-7} mg O_2/hr/spore

beech 3.4×10^{-6} mg O_2/hr/spore

average = 1.9×10^{-6} mg O_2/hr/spore

The corresponding values obtained from the same species on malt agar (4.2.6) were:

L. cymbiformis 1.7×10^{-8} mg O_2/hr/spore

T. chaetocladium 1.3×10^{-7} mg O_2/hr/spore

Thus the sporulation and respiration measurements of *L. cymbiformis* mycelial content showed a relationship closely similar to that found on malt agar. *T. chaetocladium* on kamahi also showed a similar respiration/spore value to that found on malt agar, but because that on beech was markedly higher the average figure was ten times greater than that from agar. The greater value of the respiration/spore conversion factor from beech appears to relate to low maximum spore numbers. Possibly this indicates that sporulation was not measured frequently enough to have sampled the true maximum rate.

Thus this experiment provides important support for the use of sporulation as a measure of mycelial content in naturally occurring leaves from streams, using the mycelium/spore conversion factors calculated from malt agar.

4.3 MEASUREMENT OF SPORE PRODUCTION FROM LEAVES IN STREAMS: DEVELOPMENT OF THE TECHNIQUE.

Standard conditions for measuring spore production from leaves collected from streams were chosen on the basis of some preliminary work and previously published work, the aim being to obtain as close to maximum spore production rates as possible, using similar conditions to those under which the mycelium/spore conversion factors were measured (2.1.4). In the present section a number of tests of the effect of some of the conditions chosen on the resulting spore production were made to determine which factors were most important in controlling spore production and how spore induction compares with that in agar cultures: submersion, sample weight:incubation volume ratio, length of time incubated, cutting the leaves, changing the incubation water daily, and shaking the flasks.

4.3.1 Spore production during submerged and aerial incubation of leaves from a stream.

Submersion was required for spore production by cultures of aquatic hyphomycetes. In this experiment sporulation of aquatic hyphomycetes in leaf litter from the Hakarimata stream was compared under submerged and aerial incubation to test whether submersion was of such overriding importance in natural substrates as it was in culture.

Dead leaf litter was collected in April from the Hakarimata stream and incubated either in flasks of deionised water or in flasks on damp filter paper (i.e. leaves not wet, but in a damp

atmosphere). Sporulation from the submerged leaves was measured every day, and the incubation water replaced daily, as in the standard method (2.1.4). Sporulation was measured from the aerial leaves at the end of the incubation period by adding 150 ml deionised water, then shaking the flask, and filtering a sample as for the standard method. The experiment was repeated once. In the first trial the leaves were incubated for two days. The results are summarised in Table 4.3.1a.

Table 4.3.1a: Comparison between the spore production from litter incubated submerged in deionised water or in a damp air chamber. Total aquatic hyphomycete spores/g/day are given, together with the most abundant aquatic hyphomycete species (all as mean/standard deviation x 10^3).

	Incubation Method		
	Submerged		Aerial
	Day 1	Day 2	Day 1 + 2
Total spores	485/145	1731/260	572/168
L. cymbiformis	183/129	795/3	21/7
T. chaetocladium	100/59	199/77	6/2
T. marchalianum	229/99	619/206	462/145
Alatospora spp.	14/13	56/15	63/28
T. myrti	0	0	0

Most species sporulated markedly better during submerged incubation. Two species (*T. marchalianum* and *Alatospora* spp.) sporulated abundantly in air as well, in contrast to the more typical species tested here and in pure culture (4.2.1).

In the second experiment leaves were collected in June and incubated in the same way, but the submerged leaves were sampled to test sporulation after 1, 2, 4, and 5 days. Extra moist air replicates were set up to allow sporulation to be measured twice, after two days using half the replicates, and after five days using the remainder. The results are summarised in Table 4.3.1b.

Table 4.3.1b: Comparison between the spore production from litter incubated submerged in deionised water or in a damp air chamber. Total aquatic hyphomycete spores/g/day are given, together with the most abundant aquatic hyphomycete species (all as mean/standard deviation x 10^3). "L. cymb." = *L. cymbiformis*, "L. oval." =*L. ovalis* sp. ined., "T. ch." = *T. chaetocladium*, "A. pul." = *A. pulchella*, "T. mar." = *T. marchalianum*.

	Incubation Method					
	Submerged		Air	Submerged		Air
	Day 1	Day 2	1 - 2	Day 4	Day 5	1 - 5
Total	892/69	1205/310	754/75	1735/232	556/128	447/105
L. cymb.	352/1	702/114	378/102	1330/258	385/258	146/71
L. oval.	62/12	71/7	46/8	175/24	64/23	46/41
T. ch.	211/32	105/43	138/25	83/48	26/8	21/22
A. pul.	10/8	16/13	60/20	21/8	15/9	93/32
T. mar.	87/15	140/81	96/48	68/35	37/6	94/22

Most aquatic hyphomycetes showed higher sporulation in submerged conditions, as in the previous experiment, but the same few species sporulated as well, or better, in air (*A. pulchella* and *T. marchalianum*). Thus submersion was important to spore production

from leaves as from agar cultures in most species tested.

4.3.2 Effect of substrate (weight):incubation water (volume) ratio on spore production from leaves.

The volume of incubation water used could have had a marked effect on spore production, either through concentrating or diluting leaf leachates (a nutrient source), or through the effect on oxygen levels in the leaves. The effect of the substrate weight:incubation volume ratio on spore production was tested in this experiment.

Three sample sizes of randomly collected leaves (Hakarimata stream) were incubated in 150 ml deionised water, to give a range of substrate:incubation volume ratios, and the maximum sporulation rate for each was recorded. The sample sizes used were (average) 0.34g, 1.45g, and 3.04g dry weight. The results are given in Table 4.3.2.

Table 4.3.2: Effect of leaf sample weight (g dry weight) on the maximum daily rate of spore production (x 10^3 spores/g/day), when incubated in a constant 150 ml of water. Both given as mean/standard deviation.

	Sample Weight		
	0.34/0.04g	1.45/0.28g	3.04/0.42g
Sporulation	594/466	143/46	76/32

Sporulation showed a steadily increasing degree of inhibition with successive increases in the substrate:incubation volume, although the high variation, particularly with the smallest sample size,

meant that the difference was not statistically significant. A weight:volume ratio of about 0.3 g:150 ml was used as the standard in all the present work.

4.3.3 Sporulation versus time.

In order to apply the mycelium/spore conversion factors developed in section 4.2 above, maximum spore production had to be measured. To do this by measuring sporulation every day for over a week would involve a lot of unnecessary work if spore production from leaves followed a predictable pattern, with the maximum occurring after a reasonably constant length of time.

When leaf litter was collected from a stream, incubated in the standard method, and sporulation measured at intervals, a pattern of increasing spore production to a peak, followed by a decline in sporulation, normally occurred. In eight such collections from the Hakarimata stream and Reid's Ck spore numbers were high over the period Day 2 -4 or 5, with maximum spore production being recorded at Day 2 in four experiments, Day 3 in two, and Day 4 in two. There was no apparent difference between seasons or streams in the time of maximum sporulation. Individual fungal species showed slightly greater variation in the time of maximum sporulation (Day 0 -6), but no consistent differences among species could be found. A two day incubation period was selected as the standard (for the leaf bag experiments and stream survey).

On two occasions these sporulation versus time experiments were started from an estimate of in situ sporulation in Hakarimata stream (Day 0). Flasks were submerged in the stream and leaves wafted gently into them, taking care to disturb them as little as possible. The flasks were incubated partially submerged to maintain them at

the stream temperature (13 C in the first, November, experiment, and 10 C in the second, June, experiment). Spore production was measured after six hours in the first experiment, and after 24 hours in the second, and the spore production per day estimated. On returning to the laboratory the leaves were removed from each flask, washed and chopped up as usual, but not mixed between flasks. This resulted in extremely high variation between flasks, which was misleading as the differences between the individual flasks remained constant over time. The figures for total spore numbers are given in Table 4.3.3.

Table 4.3.3: Spore production per day immediately prior to collection of leaf litter (in situ), and over the following 18 or 6 day period from collections in November and June. The results are given as mean/standard deviation, spores/g/day x 10^3.

	Spore production	
	November	June
In situ	301/280	18/12
Day 1	398/369	155/197
Day 2	676/556	312/426
Day 3	672/483	309/476
Day 4	-	370/588
Day 6	276/291	135/206
Day 13	336/227	-
Day 18	182/125	-

The in-situ sporulation varied considerably between the two experiments: from 5 to 45 % of the maximum induced under laboratory conditions. This may relate at least in part to the 3 C difference in temperature between field and laboratory in the second experiment (45 %), compared to no difference in the first (5 %). It is clear however, that the maximum values recorded in the laboratory incubation are considerably greater than the rates of sporulation occurring in the field. Thus the pattern of increasing numbers seen during incubation represents an induction of sporulation like that seen when pure cultures were submerged, rather than a recovery (to previous levels of sporulation) from the disturbance of collection.

4.3.4 Effect of the amount of cutting on sporulation from leaves.

Cutting up the leaf samples before incubation allows better mixing of the samples. Michaelides and Kendrick (1978) found that slicing pine needles increased the sporulation from them as the epidermis hindered the exit of conidiophores from the leaf. Leaves in the leaf bag experiments and stream survey were thus cut up finely in order to maximise sporulation and to minimise the effect of differences in cuticle and epidermis structures between leaf types. In the present experiment the effect of cutting on sporulation from randomly collected leaf litter from Hakarimata was tested by comparing spore production from whole leaves incubated for three days in deionised water with that from leaves cut into four pieces, or into narrow strips about 5 mm in width. The results are given into Table 4.3.4.

Table 4.3.4: Spore production from leaves incubated for three days in deionised water, either whole, coarsely, or finely cut up. The results are given as mean/standard deviation (spores/g/day x 10^3).

	Amount of cutting		
	Whole leaves	Coarsely cut	Finely cut
Spores	7.8/9.2	10.5/4.1	6.5/1.8

There were no differences in the amount of sporulation between the treatments, suggesting that for the leaf types commonly present in Hakarimata the epidermis, and therefore the amount of cut edge, does not limit sporulation. There was, however, a pattern of decreasing variance with increasing cutting as a result of increased mixing of the samples between replicates. Thus it was considered important to cut leaf samples finely before measuring sporulation.

4.3.5 Effect of changing incubation water daily on spore production from leaves.

In the standard method of measuring sporulation leaves were washed, cut up, and incubated in deionised water at 13 C. The water of incubation was replaced each day, so that the solution remained clear and easy to filter and count the spores. Changing the water of incubation could induce higher sporulation if the increased sporulation seen following laboratory incubation of leaves, compared with the in-situ measurements, results from the reduction in surface contamination (silt and organic or microbial films) achieved by washing, or possibly from leaching of nutrients from the cut leaves.

In three of the sporulation versus time experiments (4.3.3) replicate sets of flasks in which the initial deionised water was

not changed during incubation were set up and compared to identical flasks in which the standard method was followed, that is, replacing the incubation water daily. The results are given in Table 4.3.5a.

Table 4.3.5a: Effect on spore production per day from leaves incubated in deionised water of replacing the water every day compared with not doing so. The experiment was repeated three times, the first and third using leaves from Hakarimata, and the second using leaves from Reid's Ck. Sporulation is given as mean/standard deviation, total spores/g/day x 103.

	Spore Production					
	Experiment 1		Experiment 2		Experiment 3	
	Unchanged	Changed	Unchanged	Changed	Unchanged	Changed
Day 1	1927/141	3701/676	118/36	100/46	1201/348	912/67
Day 2	6978/501	7861/535	170/29	205/88	1937/324	1216/305
Day 3	7736/689	5930/230	207/15	207/92	-	-
Day 4	-	-	-	-	1802/306	1784/267
Day 5	-	-	-	-	1960/859	605/113
Day 7	3342/2462	1859/496	135/36	79/33	-	-
Day 8	-	-	-	-	1120/274	427/149
Day 12	229/107	1736/762	38/20	70/18	367/123	202/69

There was no clear difference in maximum sporulation, or in the time taken to reach it, between the two methods. There was also no difference in the time taken by spore numbers to decline following the maximum. Obviously then, changing the water daily had no effect on sporulation from leaves from Hakarimata (experiment 1 and 3) or Reid's Ck (experiment 2). It is possible that different results

might have appeared with leaves from streams with higher organic levels in the water, for example, where the further washing provided by changing the water daily might still benefit sporulation.

The fact that numbers declined in both treatments suggests that the decline in spore production did not relate to the build up of inhibitory compounds (since changing the water daily would reduce the decline), or to starvation due to the loss of leachable nutrients (since not changing the water should have reduced the decline).

Spore production was measured by filtering a 10 ml sample of incubation water through a Millipore filter and counting the spores on it. Thus, in order to draw the conclusions from the data given above it was necessary to know what proportion of the spores counted on a filter after several days incubation without changing the water had been produced in the last 24 hours (which could be compared with those produced in the flasks in which the water was changed) and how many were from previous days. Some difficulty was found in establishing this proportion.

In the absence of any substrate in the water it appeared that spores could persist in solution in significant numbers for more than 24 hours. When pieces of uncolonised air-dried leaves, or sterilised (sodium hypochlorite) leaves from a stream were placed in the spore solution the spores rapidly germinated and were lost from solution, only about 5 % of the original number remaining after 6 hours. It appears in this case that abnormal behaviour occurred, perhaps in response to nutrients leaching from the leaf pieces. When an inert substrate such as pieces of water agar were used spore loss rates lay between the two earlier attempts, with about 10 % of

the spores still in solution at 24 hours. Thus it appears that the number of spores persisting from one day to the next in the unchanged flasks above were too few to affect the conclusions given above.

4.3.6 Effect of shaken compared to still incubation on spore production from leaves.

Agitation is known to increase the rate of sporulation of aquatic hyphomycetes in pure culture (Webster, 1975, Sanders and Webster, 1980). In a preliminary experiment it was found that increasing the rate at which leaves submerged in deionised water were aerated appeared to increase the rate of sporulation, although the difference could only be detected after three days. However, aeration also caused the water volume to alter quite markedly, whereas shaking the flasks did not. Aerating the flasks caused more fragmentation of the leaves and spores than did shaking. A similar fragmentation was found with fast compared with slow shaking speeds: the best sporulation occurred when the leaf pieces remained largely unmoved. For this reason slow shaking of the flasks during incubation was chosen for the standard method of inducing sporulation. Shaking also allowed the simultaneous incubation of more flasks than could be easily done while aerating them.

In this experiment the effect of this standard treatment was determined. Leaves were collected from Hakarimata stream and were incubated in deionised water, either shaken or still. Spore production was measured periodically over a 17 day period. The results are given in Table 4.3.6.

Table 4.3.6: The effect of shaking compared to still incubation on sporulation of aquatic hyphomycetes in leaves collected from Hakarimata. The spore production (spores/g/day x 103) figures are given as mean and standard deviation.

	Spore production	
	Shake	Still
Day 1	598/184	194/84
Day 2	601/195	253/37
Day 3	603/105	208/101
Day 7	508/53	57/23
Day 11	261/32	32/23
Day 17	155/92	38/20

The spore numbers recorded with shaking were greater than those from the still incubation throughout the experiment, although the difference on any day was not statistically significant (t test) because of the high variation between flasks. However, peak rates of sporulation appear to have been attained faster than in previous experiments, perhaps because rates of sporulation in the stream were already high at the time of litter collection. Thus because the difference was evident even on day one the lower numbers recorded from the still flasks may reflect merely a difference in the aquatic hyphomycete mycelium present rather than the rate at which it was sporulating. In conclusion, agitation by shaking the flasks does not appear to be of great importance to spore induction in the present work. This suggests that the amount of agitation the leaves were subjected to in the initial washing prior to submersion, and perhaps in the daily changing of the incubation water was sufficient to induce maximal rates of sporulation without additional shaking.

In conclusion: when measuring aquatic hyphomycete spore production from leaf samples collected from streams, submersion in deionised water, or similar low nutrient solution, is important, and also a low ratio of sample weight to incubation volume. Figures for spore production (spores/g/day), measured after 2 or possibly 3 days incubation under these conditions can be used to estimate the aquatic hyphomycete mycelial content of the litter using the mycelium/spore values determined earlier (4.2.1). Important further discussion on this occurs in chapter 7. Use of the same incubation temperature as was used in the determination of the mycelium/spore conversion factors, although not tested in the present work, is likely to be important also. Cutting the leaf samples is useful in that it reduces the variation between flasks. Changing the incubation water at regular intervals and shaking the flasks are not important to spore production from leaves from normal, undisturbed streams. However, changing the incubation water 24 hours before filtering to measure spore production is recommended as a standard technique simply because for many samples this results in a cleaner filter which is easier to count spores on.

CHAPTER FIVE: RESULTS III

LEAF DECAY AND AQUATIC HYPHOMYCETE FIELD ECOLOGY.

Introduction.

The two main components of this work were the leaf bag experiment and the stream survey. These served a multiplicity of aims which are outlined here under two broad headings: leaf decay and aquatic hyphomycete population dynamics. Thus the process of leaf decay in two New Zealand streams was followed, during which aquatic hyphomycete abundance was measured using the sporulation method developed in Chapter Four and discussed in Chapter Seven.

There has been little previous work on the fate of dead leaves in New Zealand streams, in particular on the role of aquatic hyphomycetes in their decay. In overseas studies it has been possible to link rates of weight loss to sources such as leaching, microbial activity, and invertebrate consumption (Petersen and Cummins, 1974). However, Winterbourn et al (1981) have called into question the importance of detrital cycles in the energy budget of many New Zealand streams. In addition to the problem of stream stability, of how much litter is retained in stream ecosystems, it has been suggested in several studies (Towns, 1976; Davis and Winterbourn, 1977; Winterbourn et al, 1981) that both invertebrate and fungal contributions to leaf weight loss in New Zealand streams differ from their Northern Hemisphere counterparts. For example, Davis and Winterbourn (1977) found very little colonisation of mountain beech leaves by aquatic hyphomycetes in a South Island stream. They suggested that leaves of the Fagaceae in general might prove to be poor aquatic hyphomycete substrates. However, one previous study (Aimer and Segedin, 1985a) found levels of aquatic

hyphomycetes in a North Island (New Zealand) stream comparable to those commonly found in overseas studies. In the present work rates of weight loss were measured for a variety of leaf types, together with some of the potential sources of weight loss (invertebrate biomass and microbial colonisation), in order to test these observations, in particular the hypothesis that detrital cycles are not as important in at least some New Zealand streams as in Northern Hemisphere streams, and that aquatic hyphomycete colonisation of and contribution to the decay of leaves was limited, particularly in respect to some leaf types, e.g. beech leaves.

The population dynamics of the aquatic hyphomycetes colonising the leaf types studied were followed for four purposes. Firstly, previous studies have differed as to whether any evidence of successional patterns during decay was found, their absence leading to the idea that aquatic hyphomycetes formed founder-controlled communities (Bärlocher, 1982a,b). It was hoped this could be clarified in the present work. Secondly, although a variety of leaf types have been tested in Northern Hemisphere studies, and little evidence of substrate preferences found for the aquatic hyphomycetes on them, New Zealand has many previously unstudied leaf types which includes such unique examples as nikau and ponga. The aquatic hyphomycete flora of a variety of leaf types was examined here. Thirdly, a number of overseas studies, and one from New Zealand (Aimer and Segedin, 1985a) have shown a seasonal pattern in spore concentration in stream water. Higher spore concentrations occurred in autumn and winter in all studies and was related to substrate levels in the streams. However, a similar seasonal pattern also appeared to occur in spore production from leaves in the New Zealand study (Aimer, 1982). Thus there appeared to be some seasonal variation in abundance unrelated to substrate levels. One of the aims of the present work was to see if this pattern could be

repeated, i.e. was a real seasonal effect, and whether the same pattern appeared in different aquatic hyphomycetes. Fourthly, a number of previous workers have noticed differences in the aquatic hyphomycete flora of streams in different climatic zones, for example temperate compared with tropical zones (Webster and Descals, 1981; Nilsson, 1964). Other studies, made within a climatic zone, have found correlations between the aquatic hyphomycete flora and riparian vegetation type, water quality factors (for example, pH), and temperature (Nilsson, 1964; Wood-Eggenschwiler and Bärlocher, 1983; Suberkropp, 1984). In the present work two streams were chosen for intensive study, and a number of other streams were sampled once only, to test for differences in distribution patterns between aquatic hyphomycetes in New Zealand streams, and to provide a data base of aquatic hyphomycete occurrence in undisturbed stream habitats for comparison in future work.

5.1: THE LEAF BAG EXPERIMENT.

Full details of the experimental set-up for the leaf bag experiment have been given in section 2.2. In summary, the leaf bag experiment ran for 18 months, from December 1982 to May 1984 in the Hakarimata stream, and from December 1982 to April 1984 in Reid's Creek. A number of measurements were made at monthly intervals: water temperature, stream discharge, and concentrations of aquatic hyphomycete spores in the stream water. The main part of the experimental work involved immersing leaves in the streams in mesh bags and sampling regularly to follow decay. Kamahi and silver beech were submerged in both streams in December 1982 (first run) and May 1983 (second run) as fresh green leaves, and were sampled at monthly intervals, with additional one and two week samples from Hakarimata during the first month. Newly fallen (senescent) kamahi

and beech (second run, Hakarimata and Reid's Ck respectively), and freshly picked green hangehange, kanono, and kauri (first run, Hakarimata) were tested only once, in one stream, and sampled at increasing intervals (1, 2, 4, 8, 16 etc weeks). Kanono and kauri were separated into young and old green leaf types. Rimu, tawa, ponga, and nikau were introduced into the Hakarimata stream in the second run as fresh green leaves, but were sampled only twice, at 6 and 12 months. Several factors were assessed for each leaf sample, namely weight loss, respiration rate, invertebrate numbers, and fungal spore production. On two occasions the fungal flora was also assessed by particle plating methods. The results of this plating work are given in the next chapter (6.2).

The results pertaining to leaf decay processes: weight loss, invertebrate colonisation, and microbial colonisation (respiration and aquatic hyphomycete abundance), are given first. These are followed by the observations on the aquatic hyphomycete flora and how individual species varied during decay, on different leaf types, in different seasons, and between the two streams. The extended distribution patterns discovered in the stream survey and associated ecological work follow in the final sections.

5.1.1 Leaf Weight Loss and Decay Pattern.

The time taken to decay varied conspicuously between leaf species, but much less so between young and old leaves, between fresh green and senescent leaves, and between the two streams. Replicate measurements of weight loss, in addition to the other factors being measured, would have required too much leaf material. However, significant differences between leaf types were distinguishable without statistical methods by their repetition in a number of successive samples. Measurements of % weight loss for all

leaf types sampled regularly (fresh green and fallen kamahi and beech, hangehange, and young and old kanono and kauri) are given in Fig. 5.1 and 5.1a. The figures for % weight loss for the remaining leaf types (tawa, rimu, nikau, and ponga) are given in Table 5.1 below.

Table 5.1: Percentage weight loss from tawa, rimu, nikau, and ponga after 6 and 12 months immersion in Hakarimata stream.

	Tawa		Rimu		Nikau		Ponga	
	6 m	12 m	6 m	12 m	6 m	12 m	6 m	12 m
Wgt loss:	37	94	40	84	38	81	25	59

Hangehange lost weight fastest, followed by kanono, then beech and kamahi, tawa, rimu, nikau, and finally ponga and kauri. Weight loss of fallen beech did not differ markedly from that of green beech until the last sample, suggesting that weight loss later in decay may have been slower in fallen beech. More samples during these stages would be required to be certain however. Fallen kamahi did not differ from green in the first half of decay (weight loss <50 %), but also appeared to be slightly slower decaying in the second half. Thus it appears senescent (fallen) leaves may decay slightly more slowly than green leaves, the difference becoming apparent in the second half of decay.

There were only slight differences between young and old leaf types. Old kanono differed noticeably from young kanono only in the persistence of the last leaf fragments, although the results for young kanono fluctuated widely during the first two months. Weight loss in young kauri leaves lagged slightly behind that of old kauri leaves from two months on, but the difference was probably not Fig

Fig. 5.1: Percent weight loss from leaves in the Hakarimata stream during the leaf bag experiment: fresh kamahi and beech (first run leaves (1) introduced into the stream in December 1982 = 0 months, and second run leaves (2) in May 1983 = 5 months), hangehange, young and old kanono, young and old kauri, and fallen (senescent) kamahi leaves, measured after 1 and 2 weeks, then at monthly or greater intervals.

Fig 5.1

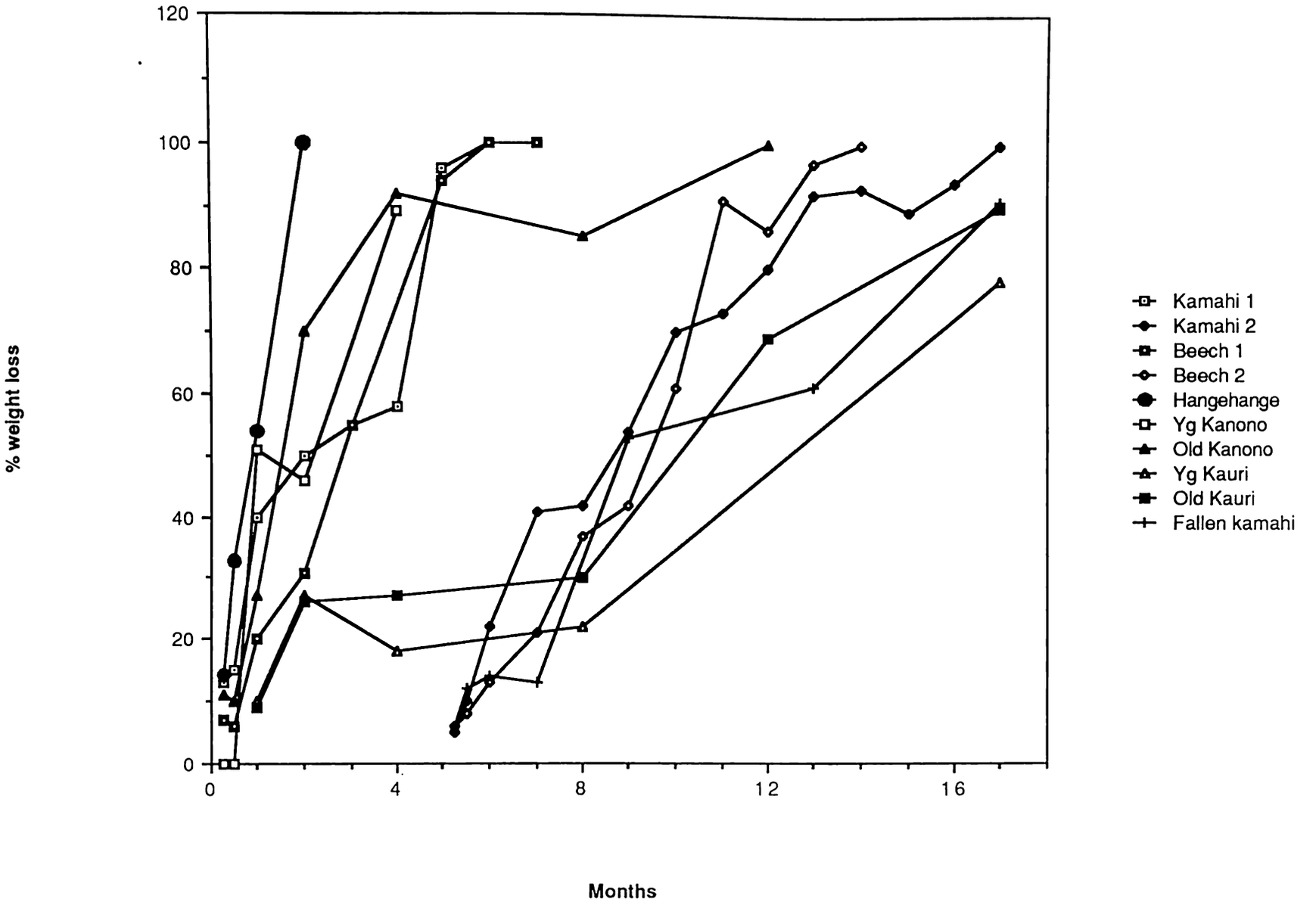
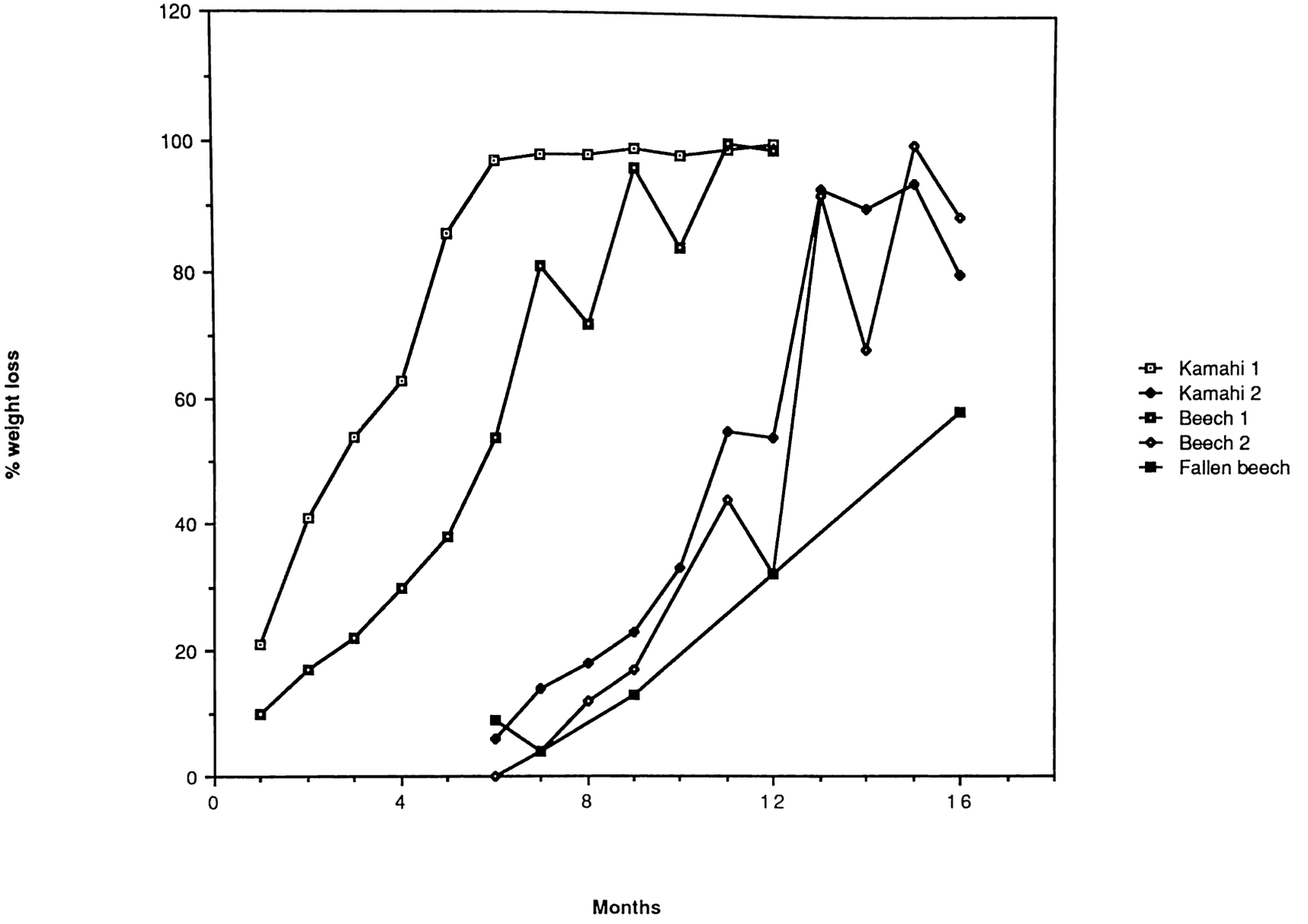

120
100
80
60
40
20
0
0
4
8
12
16
% weight loss
Months
Kamahi 1
Kamahi 2
Beech 1
Beech 2
Hangehange
Yg Kanono
Old Kanono
Yg Kauri
Old Kauri
Fallen kamahi

Fig. 5.1a: Percent weight loss from leaves in Reid's Ck during the leaf bag experiment: fresh kamahi and beech (first run leaves (1) introduced into the stream in December 1982 = 0 months, and second run leaves (2) in May 1983 = 5 months), and fallen (senescent) beech, measured at monthly or greater intervals.

5.1a


120
100
80
60
40
20
0
0
4
8
12
16
% weight loss
Months
Kamahi 1
Kamahi 2
Beech 1
Beech 2
Fallen beech

significant as only three samples of both leaf types were taken after this time, and the difference between the leaf types did not continue to increase.

Differences between streams and seasons appeared in the data for kamahi and beech. Weight losses occurred slightly faster in Hakarimata than Reid's Ck, and also in the first run (beginning in summer) compared with the second (beginning in winter).

Within a leaf type the decay rate followed a distinct pattern, most clearly visible in the leaf types sampled at monthly intervals. Rates of weight loss with time (g/100g/month) for kamahi and beech are shown in Fig. 5.2 and 5.3. The basic pattern was one of an initially high rate of weight loss, a subsequent steady decline, then a rise to a second peak at about 4 -5 months, after which it declined again. The same basic pattern appeared in the results for both leaf types, although for kamahi compared with beech the initial peak in decay rate tended to be proportionately more important to total weight loss than the second. In the second run (winter) in Reid's Ck there was a lag before the initial high rate was achieved.

Differences in the pattern of decay were visible between leaf types, and to a lesser extent between streams and runs (i.e. seasons). Detailed descriptions of the appearance of the leaves during decay for each leaf type are given in Appendix 2. In summary: hangehange rapidly became slimy and disintegated; young kanono initially followed the same pattern, but a vein network persisted long after the interveinal tissue had collapsed and worn away, or been consumed; old kanono and kamahi (in the first run) showed a similar pattern of loss of interveinal tissue before the veins, but slightly slower; in kamahi (second run), beech, and kauri the leaf persisted in entire form for longer, becoming dark and brittle, and the veins and interveinal tissue were generally Fig

Fig. 5.2: Rate of weight loss (g/100g/month) of kamahi in Hakarimata and Reid's Ck (first run leaves (1) introduced into the streams in December 1982 = 0 months, and second run leaves (2) in May 1983 = 5 months).

Fig 5.2

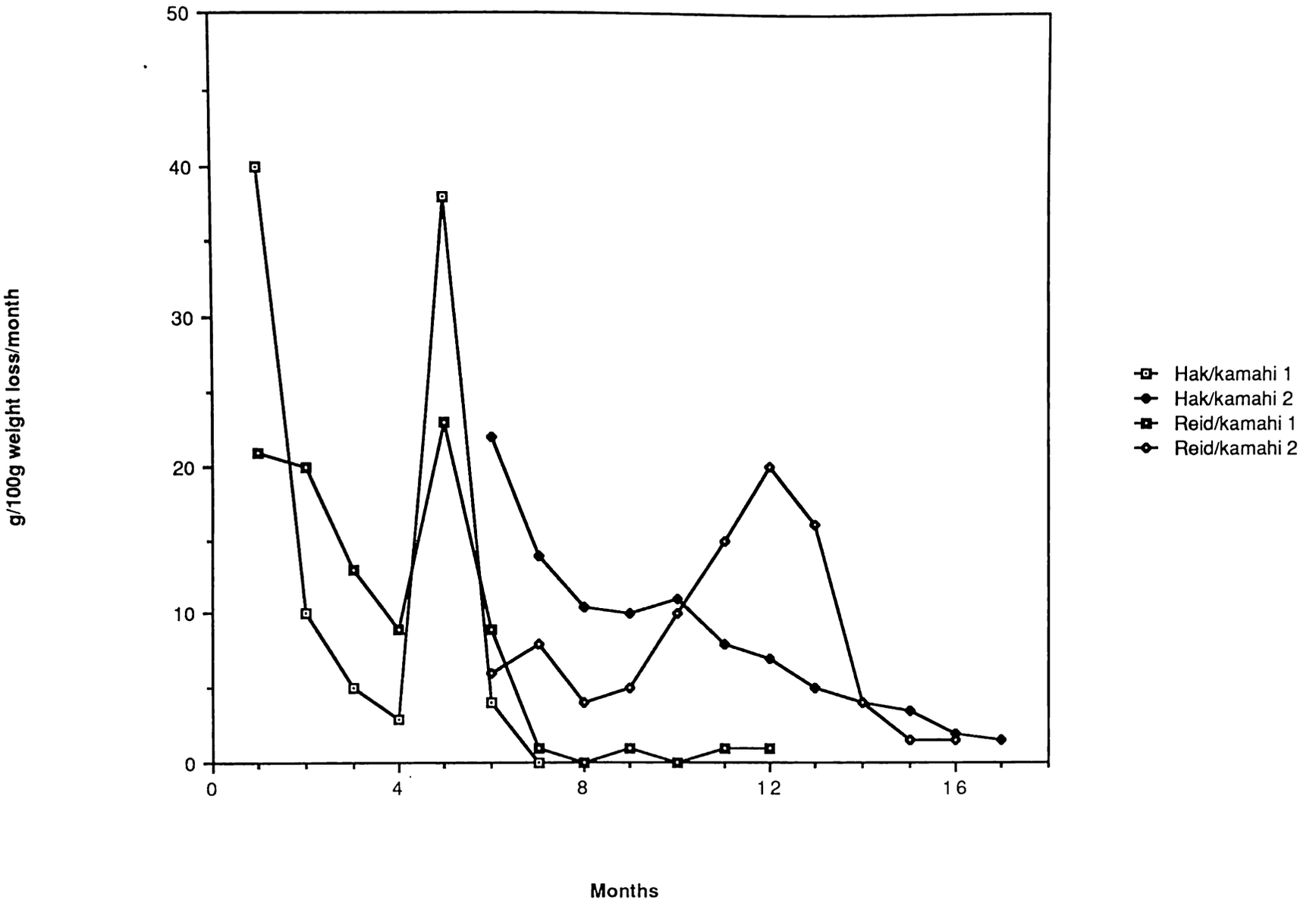
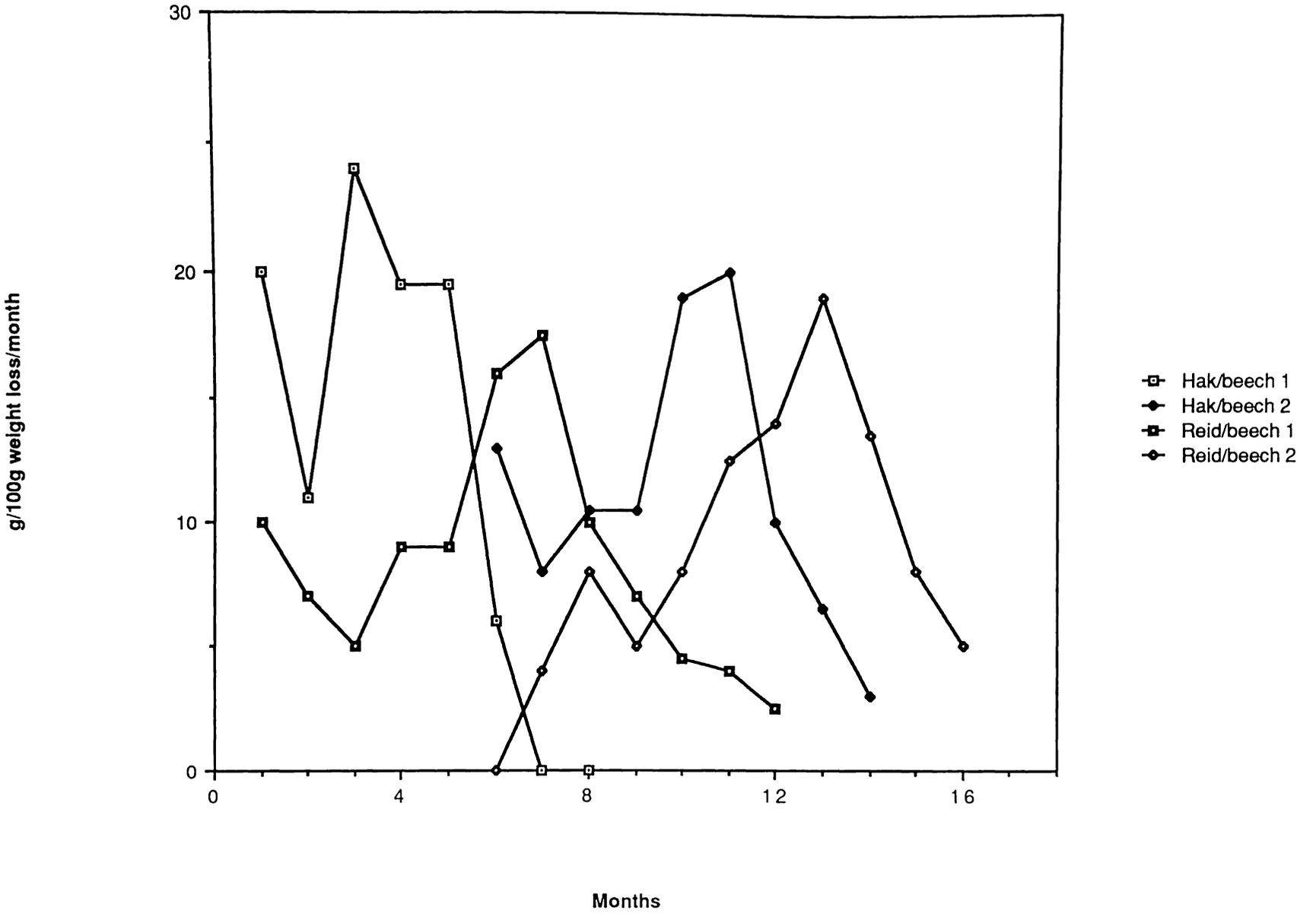

50
40
30
20
10
0
0
4
8
12
16
g/100g weight loss/month
Months
Hak/kamahi 1
Hak/kamahi 2
Reid/kamahi 1
Reid/kamahi 2

Fig. 5.3: Rate of weight loss (g/100g/month) of beech in Hakarimata and Reid's Ck (first run leaves (1) introduced into the streams in December 1982 = 0 months, and second run leaves (2) in May 1983 = 5 months).

Fig 5.3


30
20
10
0
0
4
8
12
16
g/100g weight loss/month
Months
Hak/beech 1
Hak/beech 2
Reid/beech 1
Reid/beech 2

consumed or fragmented together.

Weight loss can be attributed to physical processes, invertebrate consumption, and microbial utilisation. Invertebrate biomass and microbial colonisation are given in the next few sections.

5.1.2 Invertebrates.

Invertebrates belonging to a number of orders were seen (Appendix 3). No attempt was made to identify the invertebrates, or to assign them to feeding categories. Thus the presence of invertebrates does not necessarily imply active consumption of the leaves by all of them, but it is assumed that leaf types or periods of decay showing high invertebrate colonisation are likely to have corresponded to higher consumption than when invertebrate numbers were low.

Observations were made of the numbers and size of invertebrates living in the leaf bags. By assigning arbitrary values of 1, 2, and 3 to "small", "medium", and "large" sized invertebrates a rough, relative biomass indicator was obtained. Invertebrate "biomass" is given for all leaf types in Tables 5.2 and 5.3, together with biomass divided by the dry weight of leaf material remaining in the bag on sampling. The figures for biomass/g leaf material became increasingly unreliable late in decay when only small amounts of leaf material remained, and some invertebrates occurred in completely empty bags. However the figures were useful for comparing leaf types with very different sample sizes before they became too well decayed. It is important to note that there was a gap of several hours between collection of the mesh bags and their separation from each other, during which time some invertebrates

Table 5.2: Invertebrate biomass indices calculated for each leaf type at each collection date from the Hakarimata stream by summing the biomass values of invertebrates assigned to size classes given the arbitrary values of 1, 2, or 3. The leaf types used were fresh kamahi, beech, hangehange, young and old kanono, and young and old kauri, all introduced in the first run (December), and fresh kamahi, beech, tawa, nikau, ponga, rimu, and fallen (senescent) kamahi, introduced in the second run (May). The values given in brackets are the biomass indices divided by the leaf sample weight. "NS" = not sampled.

	First Run Leaves							Second Run Leaves					
Date	Kamahi	Beech	Hange.	yg Kan.	old Kan.	yg Kau.	old Kau.	Kamahi	Beech	f. Kamahi	Tawa	Nikau	Ponga	Rimu
21/12/82	18 (7)	2 (2)	4 (4)	4 (2)	2 (1)	0 (0)	2 (1)							
28/12/82	2 (1)	2 (2)	1 (1)	2 (1)	12 (3)	4 (3)	4 (2)							
15/1/83	32 (19)	3 (4)	7 (14)	10 (7)	10 (3)	4 (3)	NS							
17/2/83	6 (4)	8 (13)	5 (45)	10 (8)	10 (4)	2 (1)	3 (1)							
18/3/83	33 (29)	20 (50)												
18/4/83	15 (13)			18 (66)	18 (61)	10 (9)	2 (1)							
14/5/83	17 (17)	0												
22/5/83								0 (0)	3 (4)	0 (0)				
27/5/83								0 (0)	2 (3)	NS				
13/6/83	3 (1000)	4 (1000)						10 (3)	6 (3)	22 (4)				

Table 5.2 cont.

Date	Kamahi	Beech	Hange.	yg Kan.	old Kan.	yg Kau.	old Kau.	Kamahi	Beech	f. Kamahi	Tawa	Nikau	Ponga	Rimu
15/7/83	13(6500)	NS						24 (10)	23 (34)	40 (7)				
13/8/83				9 (22)	35 (61)	9 (7)	7 (3)	48 (21)	32 (61)					
16/9/83								56 (29)	19 (38)	86 (27)				
15/10/83								41 (34)	12 (36)					
15/11/83								44 (38)	12 (158)		25 (11)	30 (12)	49 (15)	19 (9)
18/12/83				4 (800)	1 (62)	NS	24 (21)	NS	NS					
16/1/83								24 (69)	2 (77)	59 (22)				
14/2/83								18 (65)						
12/3/83								34 (75)						
11/4/83								17 (72)						
11/5/83								17 (69)		7 (NS)				

Table 5.3: Invertebrate biomass indices calculated for each leaf type at each collection date from Reid's Ck by summing the values of invertebrates assigned arbitrary biomass values of 1, 2, or 3. The leaf types used were fresh kamahi and beech in the first run (leaves introduced in December), and fresh kamahi, fresh beech, and fallen (senescent) beech in the second run (introduced in May). Figures for biomass indices divided by the leaf sample weights are given in brackets. "NS" = not sampled; "-" = invertebrates present but no leaf material.

	First Run		Second Run		
Date	Kamahi	Beech	Kamahi	Beech	Fallen Beech
8/1/83	2 (1)	4 (5)			
8/2/83	8 (4)	2 (3)			
14/3/83	21 (16)	NS			
13/4/83	6 (6)	0			
11/5/83	NS	1 (2)			
6/6/83	34 (382)	29 (71)	5 (2)	4 (3)	5 (3)
8/7/83	12 (179)	8 (48)	NS	8 (10)	5 (3)
7/8/83	6 (113)	5 (20)	34 (11)	2 (3)	
14/9/83	26 (1300)	10 (256)	18 (6)	6 (9)	8 (6)
14/10/83	9 (167)	19 (137)	54 (21)	9 (14)	
11/11/83	9 (1286)	0	55 (32)	13 (27)	
11/12/83	NS	NS	NS	NS	NS
8/1/84			51 (198)	8 (123)	
11/2/84			20 (51)	1 (4)	
11/3/84			11 (50)	12 (-)	
7/4/84			12 (16)	1 (11)	NS

could have moved between the bags.

Invertebrate biomass in the mesh bags showed distinct changes with time, which varied between seasons and between some leaf types. In the Hakarimata stream all leaf types showed a similar pattern of initially increasing invertebrate biomass, reaching a plateau phase, then slowly declining. All leaf types attained a roughly similar biomass value in the plateau phase, with the exception of hangehange and young kanono, which bore significantly lower biomass.

The different leaf types varied in the time taken to reach the plateau, correlating roughly with the time taken to decay by the leaf types. Invertebrate biomass on kamahi and beech (and probably old kanono) reached the plateau value at 3 months in the first run, and at 3 to 4 months in the second run, just preceding the peak in rate of decay (Fig. 5.2 and 5.3). Invertebrate biomass on fallen kamahi also reached a peak at 4 months. In kauri the first observed record of the plateau value biomass occurred at 12 months, in the last sample for which invertebrates were recorded, and coincided with the period in which decay became visible. Although a plateau value was not demonstrated for tawa, nikau, ponga, and rimu, the biomass/g values obtained at 6 months correspond better with those obtained from kamahi at the same stage of decay (% weight loss) than those obtained after the same length of time in the stream.

Patterns within Reid's Ck were less clear, the plateau phase being more irregular than in Hakarimata, possibly because of the longer lag between removal of the samples from the stream and their processing.

5.1.3 Microbial Respiration Rates.

The respiration rates of the leaves during decay were obtained to provide a measure of total microbial colonisation.

Respiration rate was measured from whole, rinsed leaves immersed in stream water, at 13 C, as soon after collection as possible (2.2.3). The results for all leaf types are given in Tables 5.4 and 5.5 and the results for kamahi and beech shown in Figs 5.4 -5.11. Very few measurements were made in the later stages of decay as insufficient leaf material remained.

A consistent pattern with time could be seen in the results for kamahi and beech. Respiration reached a peak in the first run at 3 months in both streams. The same peak was apparent in Reid's Ck in the second run, but was less clearly defined in Hakarimata. Thus respiration reached a peak either prior to (second run beech), or close to 50 % weight loss. This coincided with a low point in decay rate (Figs 5.2 and 5.3) in most cases. Direct microbial consumption cannot therefore be the major source of weight loss in kamahi and beech. In comparison, invertebrate biomass indices remained high for several months after the peaks in respiration rate, suggesting that the potential for weight loss through invertebrate consumption was greater later in decay.

Quantitative differences in respiration rate were apparent between kamahi and beech, and betweeen the first (summer) and second (winter) runs. Thus respiration rates were higher in summer than winter on both leaf types. The two leaf types differed only in summer, when kamahi leaves appeared to respire faster than beech, coinciding with a more soft-rotting decay pattern in first run kamahi.

Respiration rates on the remaining leaf types varied, with species with faster initial decay rates and soft-rot decay patterns

Table 5.4: Respiration rate of leaf material immersed in the Hakarimata stream in December (first run) or May (second run) at each collection date (mg O_2/g/hr). The leaf types used in the first run were fresh kamahi, beech, hangehange, young kanono, old kanono, young kauri, and old kauri. Fresh kamahi, beech, tawa, nikau, ponga, rimu, and fallen (senescent) kamahi were used in the second run.

	First Run Leaves							Second Run Leaves						
Date	Kamahi	Beech	Hange.	yg Kan.	old Kan.	yg Kau.	old Kau.	Kamahi	Beech	f. Kam.	Tawa	Nikau	Ponga	Rimu
21/12/82	>0.117	0.098	0.021	>0.088	>0.057	0.044	0.023							
28/12/82	0.275	0.034	0.247	0.168	0.158	0.071	0.034							
15/1/83	0.272	0.186	0.395	0.796	0.326	0.072	0.069							
17/2/83	0.189	0.214	-	0.440	0.321	0.084	0.040							
18/3/83	0.448	0.209												
18/4/83	0.037	-		-	0.373	0.005	0.039							
14/5/83	0.060	-												
22/5/83	-	-						0.067	0.038	0.091				
27/5/83	-	-						0.094	0.053	0.090				
13/6/83	-	-						0.054	0.164	0.118				
15/7/83		-						0.116	0.108	0.064				
13/8/83				0.110	0.162	0.054	0.048	0.117	0.064					
16/9/83								0.153	0.066	0.088				
15/10/83								0.044	0.060					
15/11/83								0.039	-		0.127	0.085	0.021	0.060
18/12/83						-	0.046	0.062	0.076					
12/3/84								0.057						

Table 5.5: Respiration rates of leaves immersed in Reid's Ck in December (first run) and May (second run) at each collection date (mg O_2/g/hr). The leaf types used in the first run were fresh kamahi and beech, and in the second run were fresh kamahi, beech, and fallen (senescent) beech.

	First Run		Second Run Leaves		
Date	Kamahi	Beech	Kamahi	Beech	f. Beech
8/1/83	0.205	0.042			
8/2/83	0.033	0.015			
14/3/83	0.352	0.123			
13/4/83	0.218	0.097			
11/5/83	0.248	0.090			
6/6/83	0.193	0.118	0.020	0.040	0.107
8/7/83	0	0	0.054	0.033	0.022
7/8/83	0.044	0.008	0.095	0.101	
14/9/83	NS	NS	0.069	0.094	0.037
14/10/83	0.192	0.055	0.053	0.085	
11/11/83			0.066	0.065	
11/12/83			0.041		0.035
11/3/84			0.065		

tending to show higher and earlier peaks in respiration rate. Thus hangehange and young kanono reached peak respiration rates in the first month which were relatively high compared with the other leaf types. Kauri leaves showed maximum respiration rates after 1 -2 months, but generally rates were rather low and unvarying throughout the experiment.

5.1.4 Sporulation as a Measurement of Aquatic Hyphomycetes: Total Spore Production and Number of Species.

The respiration rates measured above could have derived from a number of microbial groups. The presence in the leaves of aquatic hyphomycetes and other conidial fungi which sporulate in submerged conditions was measured separately through the number of spores produced. High levels of aquatic hyphomycete colonisation would suggest that these fungi contributed significantly to the decay of the particular leaf type, while low levels, as indicated by low spore production and few species detected, would indicate that the leaf type was a poor substrate for aquatic hyphomycetes. In addition to the role of aquatic hyphomycetes in the leaf decay scheme, and their substrate preferences, the data will also show any seasonal pattern in aquatic hyphomycete abundance within the leaf material, similar to that found by Aimer (1982). Differences between the streams could also be expected if variation in the potential for a detrital cycle, independent of the retentiveness of the stream, exists among stream types. Fresh green leaves were used in the present work, in contrast to the senescent and/or air-dried leaves used by most previous workers. The effect of these different conditions on colonisation by aquatic hyphomycetes was also tested here, in order to be able to compare the results with those from

other workers. These aspects are treated in separate subsections.

Total spore production from each sample for each leaf type in the leaf bag experiment is given in Table 5.6. The average spore production figures for each leaf type for the whole period are given in Table 5.7/7a. Total spore production from kamahi and beech are also shown in Figs 5.4 -5.11. The number of species recorded are given below in section 5.1.4a.

5.1.4a Differences between leaf types.

Total spore production from all leaf types, in both streams and both runs (Figs 5.4 -5.11, Tables 5.6), was initially low but rose rapidly in most cases, to a peak, and declined towards the end of decay. The different leaf types varied in the time taken to reach peak sporulation rates, the size of the peak (Table 5.6) and average (Table 5.7/7a, -pp.198-201) spore production observed, and in the number of species recorded. As a result, the leaf types could be divided into three groups:

1) Hangehange and young kanono were rapidly colonised, spore production reaching a peak after one month, but both peak and average sporulation rates recorded were low in contrast to the high respiration rates recorded (5.1.3). Only 12 and 14 aquatic hyphomycete species were recorded on hangehange and young kanono respectively.

2) Old kanono, kamahi, and beech were more slowly colonised, reaching greater peak and average rates of spore production, and being colonised by more species. Twenty three species were recorded on kamahi (first run, Hakarimata), 18 on beech (first run, Hakarimata), and 22 on old kanono.

3) Young and old kauri were both very slowly colonised, but eventually reached peak sporulation rates not very different from kamahi and beech. The total numbers of species seen were closely

Table 5.6: Total number of spores produced per day after two days incubation of leaf samples from the Hakarimata stream and Reid's Ck at each sampling date (mean x 10^3 spores/g/day, followed by the standard deviation on the next line, after "/". The leaves were introduced to the streams during December (first run) and May (second run). The leaf types used were fresh kamahi, beech, hangehange, young kanono, old kanono, young kauri, old kauri, tawa, nikau, ponga, and rimu, and fallen (senescent) kamahi and beech.

	Sampling Date.																			
	1 wk	2 wk	Jan.	Feb.	Mar.	Apr.	May	1 wk	2 wk	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
HAKARIMATA																					
kamahi 1	37	46	223	269	1230	810	129			834	806										
	/7	/20	/165	/176	/387	/109	/38			/118	/222										
kamahi 2								0	2	36	365	5513	2139	228	80	18	14	4	4	1	2
								/0	/1	/18	/119	/521	/757	/69	/33	/10	/7	/1	/1	/0	/-
beech 1	7	7	3032	4124	407	347	255			103		8									
	/10	/2	/2258	/1149	/214	/52	/51			/31		/-									
beech 2								0	1	355	2014	7261	1370	266	19	12	4	2			
								/1	/2	/194	/775	/2102	/295	/44	/9	/-	/-	/-			
fallen k.								13	42	631	3957		962				3				2
								/5	/37	/328	/1546		/661				/-				/1

Table 5.6 cont.

	1 wk	2 wk	Jan.	Feb.	Mar.	Apr.	May	1 wk	2 wk	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
hange.	25	5	29																		
	/3	/4	/28																		
yg kan.	0	0	6	64		21															
	/0	/0	/2	/61		/31															
old kan.	0	10	275	34		159						331				40					
	/0	/2	/218	/31		/73						/383				/-					
yg kau.			0	19		507						1180				282					550
			/1	/22		/318						/202				/88					/256
old kau.			0	108		1020						2004				138					71
			/0	/76		/645						/606				/52					/7
tawa															574						7
															/116						/-
nikau															3						11
															/0						/6
ponga															35						67
															/14						/2
rimu															411						3
															/62						/1

Table 5.6 cont.

	1 wk	2 wk	Jan.	Feb.	Mar.	Apr.	May	1 wk	2 wk	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
REID'S CK																					
kamahi 1			27	149	751		644			1168	1102	349	110	351	473	299					
			/8	/52	/25		/20			/712	/131	/-	/-	/-	/-	/-					
kamahi 2										7	164	1046	498	929	386	614	311	113	73	25	
										/-	/121	/261	/44	/37	/51	/80	/52	/28	/-	/-	
beech 1			1	60	637	527	584			985	500	76	745	840	137	19					
			/0	/18	/18	/147	/131			/274	/66	/2	/354	/138	/40	/-					
beech 2										1	41	255	828	559	164	553	229	127	200	221	
										/1	/9	/71	/25	/46	/8	/23	/59	/25	54	/60	
fallen b.										15	13		103			98				29	
										/6	/8		/60			/18				/4	

• + 181 ascospores.

similar to those recorded on kamahi and beech: 20 on young and 18 on old kauri. Thus, despite the low respiration rates recorded for kauri it was well colonised by aquatic hyphomycetes.

There was only a rough indication of the level of colonisation (spore production and number of species) of rimu, tawa, nikau, and ponga compared with the above types as they were sampled only twice. On the basis of these few samples rimu and tawa appeared to reach spore production figures about the middle of decay that were within the range seen on kamahi and beech at a comparable stage of decay, and the total numbers of species (20 on rimu and 21 on tawa) are similar to those of kamahi and beech immersed at the same time (25 and 22 on second run leaves in Hakarimata). Nikau and ponga, in contrast, show very low spore numbers for a comparable state of decay and fewer species (only 12 on nikau and 11 on ponga) than rimu or tawa. Large numbers of ascospores were seen in the 12 month sample of nikau and 90% of the other spores present did not belong to known aquatic hyphomycete species. Conidia not recognisable as aquatic hyphomycetes were also prominant on ponga. Obviously then, nikau and ponga were significantly poorer substrates for aquatic hyphomycetes generally than kamahi or beech, and other microorganisms were presumably more important in their decomposition.

5.1.4b Condition of leaves entering stream.

Fresh green leaves were used predominantly in the present work as they were easier to obtain in sufficient quantities in summer and winter than senescent leaves, and would compose at least a minor part of the leaf litter normally entering a stream. Kamahi and

beech were tested once as naturally senescent (fallen) leaves, corresponding to the normal state in which most leaves would enter the streams (Table 5.6). These were collected immediately prior to use, either from the tree or under it, while still yellow. Both fallen leaf types were initially colonised faster than green leaves. Peak and average spore production together with number of species seen (23) were very similar in both types of kamahi leaves. However, fallen beech leaves showed conspicuously lower spore numbers, both peak and average, than green beech, and fewer species (19 compared with 30 in second run beech in Reid's Ck). These results may relate to the infrequent sampling intervals compared to green beech, particularly during the period when Reid's Ck temperatures rose to those of Hakarimata (in which fallen kamahi was tested), so that the high sporulation expected in the middle of decay was not sampled. Thus there is no firm evidence that green leaves (used in most of the leaf bag experiment) behave differently from fallen leaves (which would normally be more abundant in streams).

Previous studies have generally used air dried leaves, either senescent or green, to study leaf decay in streams. Fresh leaves were used in the present work because they were more natural to the habitat. In order to test the effect of this drying treatment air-dried green kamahi leaves (Table 5.8) were placed in both streams at intervals, and sampled after one month. This also allowed an examination of the effect of season on colonisation rate, independent of the rate of death of the living leaf types.

Table 5.8: Weight loss (%), total spore production (mean/standard deviation x 10^3 spores/g), and number of species on samples of air dried kamahi leaves following immersion in Hakarimata and Reid's Ck for one month prior to the dates stated.

	Month Removed				
	Aug.	Nov.	Feb.	Mar.	June
HAKARIMATA					
% wgt loss:	30	0?	92	57	22
Spores/g:	827.9	1623.4	8180.5	6594.0	1977.7
(x 10^3)	/244.3	/772.0	/534.8	/2396.0	/1410.5
No. species:	8	13	7	6	7
REID'S CK					
% wgt loss:	15	11		22	
Spores/g:	20.2	-		126.5	
(x 10^3)	/3.5			/60.3	
No. species:	6	-		12	

In Hakarimata dried kamahi leaves immersed in summer rotted faster than fresh kamahi (up to 92 % weight loss in one month) but were very heavily colonised (spore production higher than recorded from kamahi at any time). In winter and spring the rate of weight loss was slower, similar to that of fresh kamahi, but aquatic hyphomycete colonisation was more advanced, being equivalent to that on fresh kamahi after 2 -3 months, although lower than that of dried kamahi in summer. Although fewer samples were incubated in Reid's Ck, there appeared to be less difference in rate of weight loss between seasons from dried kamahi. In summer the rate was comparable to that of fresh leaves, while in winter it was about one

month faster than second run kamahi. Aquatic hyphomycete colonisation was about one month more advanced than comparable fresh kamahi in all seasons. Thus there were noticeable differences in decay rate and aquatic hyphomycete spore production between dried and fresh kamahi.

Dried kamahi appeared to be a better substrate for aquatic hyphomycetes than fresh, even when the decay rate was comparable to, or even faster than the fastest decaying leaf types (group one above). This suggests that some factor other than decay rate, although frequently coincident with it, must determine the suitability of a particular leaf type for aquatic hyphomycete colonisation, at least in part. There was a visible difference in the pattern of decay between the group one leaves and dried kamahi: kamahi was usually brown rather than olive brown, did not go as mushy but was more fragmented and chewed, being well colonised by invertebrates. Thus the factors, possibly biochemical, determining the leaf decay pattern may determine the suitability of the substrate for aquatic hyphomycetes.

5.1.4c Relationship between spore production, leaf weight loss, respiration, and invertebrate biomass.

The relationship between spore production and leaf weight loss was examined more closely in the case of kamahi and beech. Peak spore numbers on kamahi were recorded at 3 months in both streams and runs except for the first run in Reid's Ck (7 months) (Figs 5.4, 5.6, 5.8, and 5.10), while on beech peak spore numbers occurred at 2 -4 months (Figs 5.5, 5.7, 5.9, and 5.11), like kamahi, except for the first run in Reid's Ck (6 months). Thus peak spore production coincided with about 20 -50 % weight loss of the substrate in most

Fig. 5.4: Weight loss (%), spore production (x 10^4 spores/g), and respiration rate (x 10^{-2} mg/g/hr) measured from kamahi leaves initially introduced into the Hakarimata stream in December and subsequently sampled after 1 and 2 weeks and then at monthly intervals. (Note: first respiration measurement unclear but apparently greater than all subsequent values.)

Fig. 5.5: Weight loss (%), spore production (x 5 x 10^4 spores/g), and respiration rate (x 10^{-2} mg/g/hr) measured from beech leaves initially introduced into the Hakarimata stream in December and subsequently sampled after 1 and 2 weeks and then at monthly intervals.

Fig 5.4

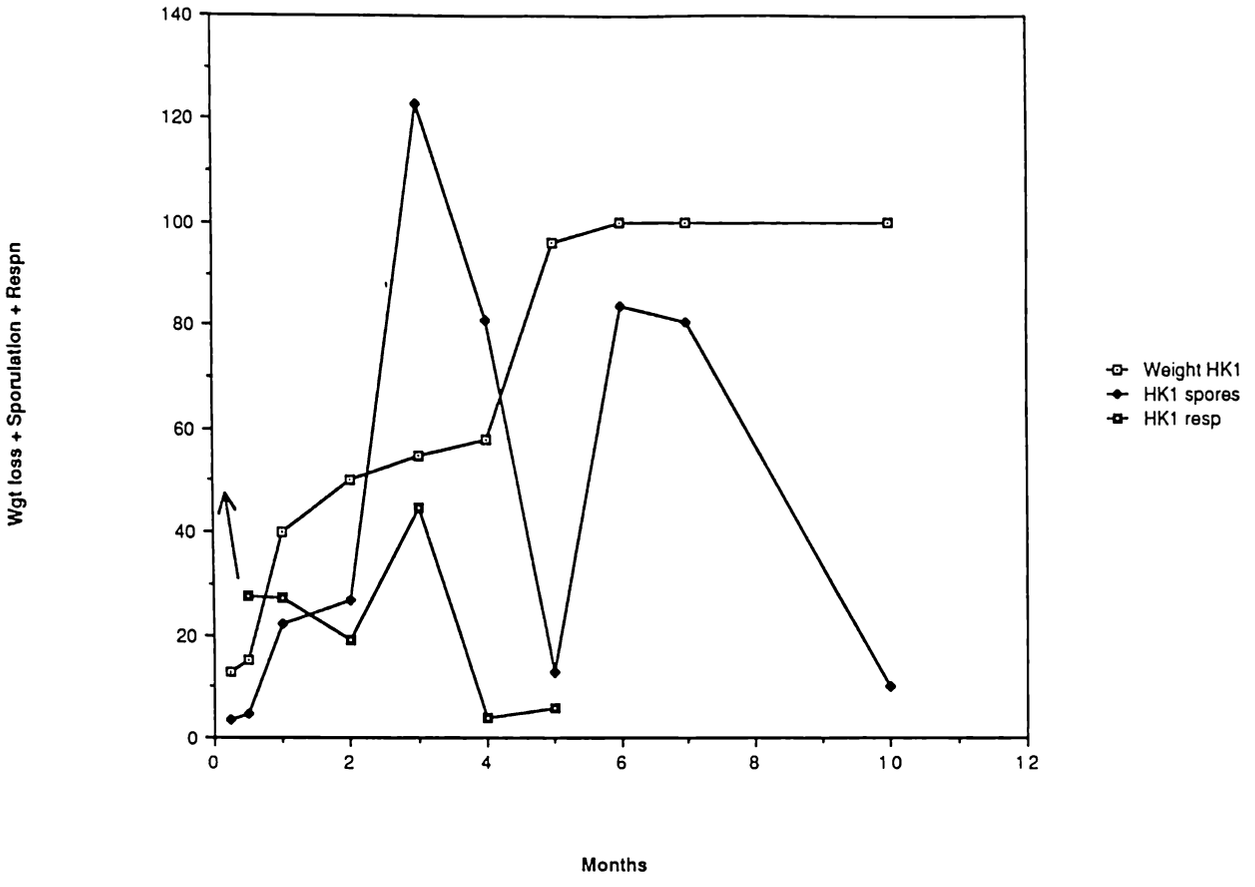

Wgt loss + Sporulation + Respn
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
Months
Weight HK1
HK1 spores
HK1 resp

Fig 5.5

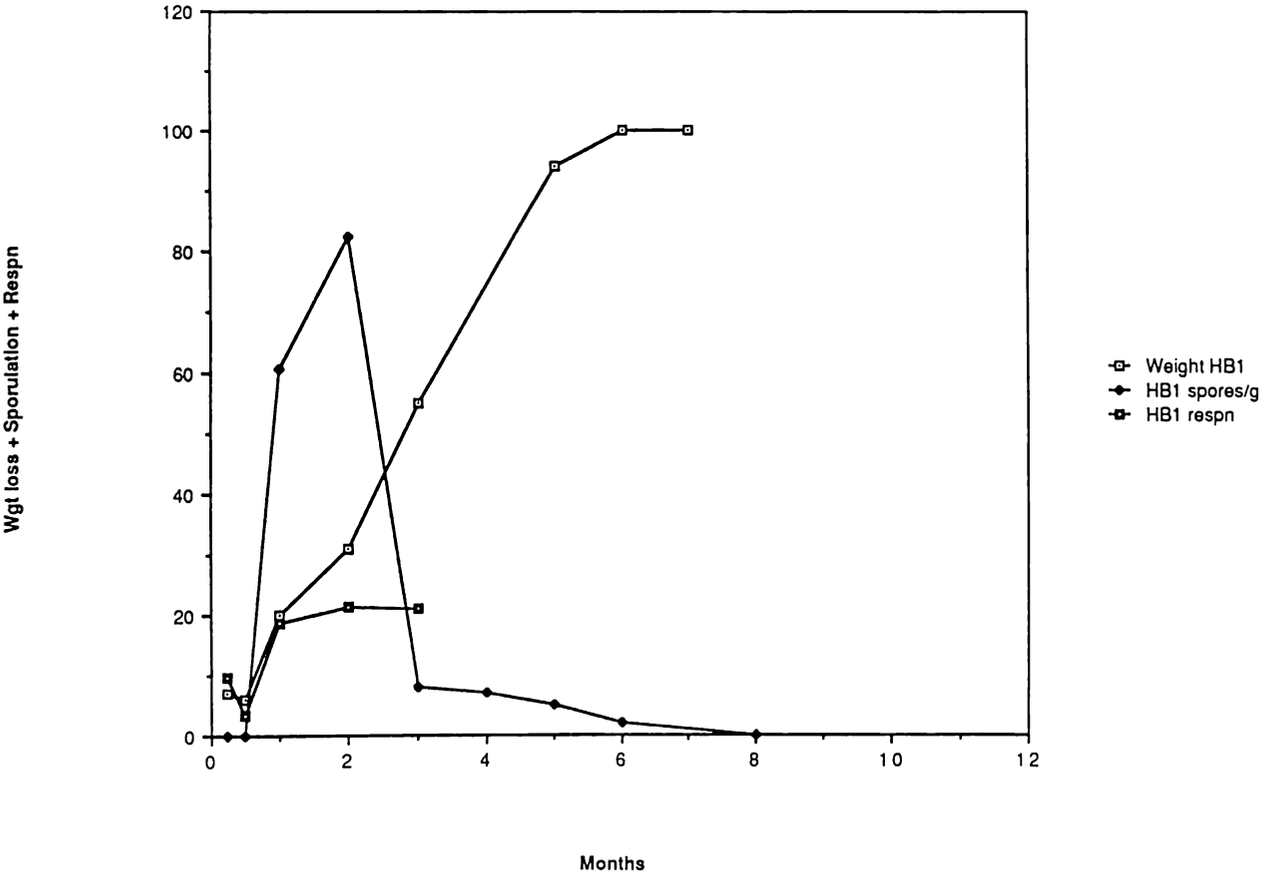

Wgt loss + Sporulation + Respn
120
100
80
60
40
20
0
0
2
4
6
8
10
12
Months
Weight HB1
HB1 spores/g
HB1 respn

Fig. 5.6: Weight loss (%), spore production (x 5 x 10^4 spores/g), and respiration rate (x 0.25 x 10^{-2} mg/g/hr) measured from kamahi leaves initially introduced into the Hakarimata stream in May and subsequently sampled after 1 and 2 weeks and then at monthly intervals.

Fig. 5.7: Weight loss (%), spore production (x 5 x 10^4 spores/g), and respiration rate (x 0.25 x 10^{-2} mg/g/hr) measured from beech leaves initially introduced into the Hakarimata stream in May and subsequently sampled after 1 and 2 weeks and then at monthly intervals.

Fig 5.6

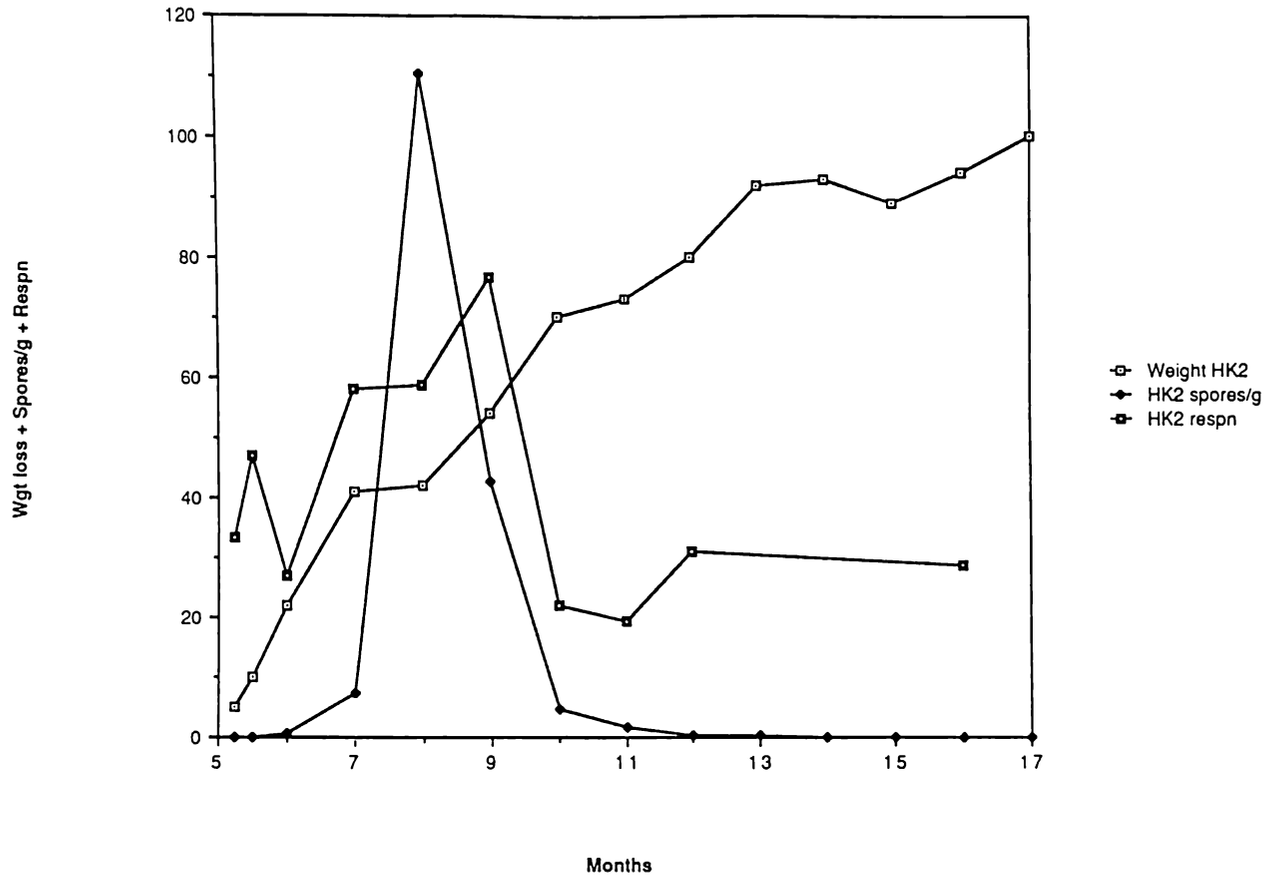

120
100
80
60
40
20
0
5
7
9
11
13
15
17
Wgt loss + Spores/g + Respn
Months
Weight HK2
HK2 spores/g
HK2 respn

Fig 5.7

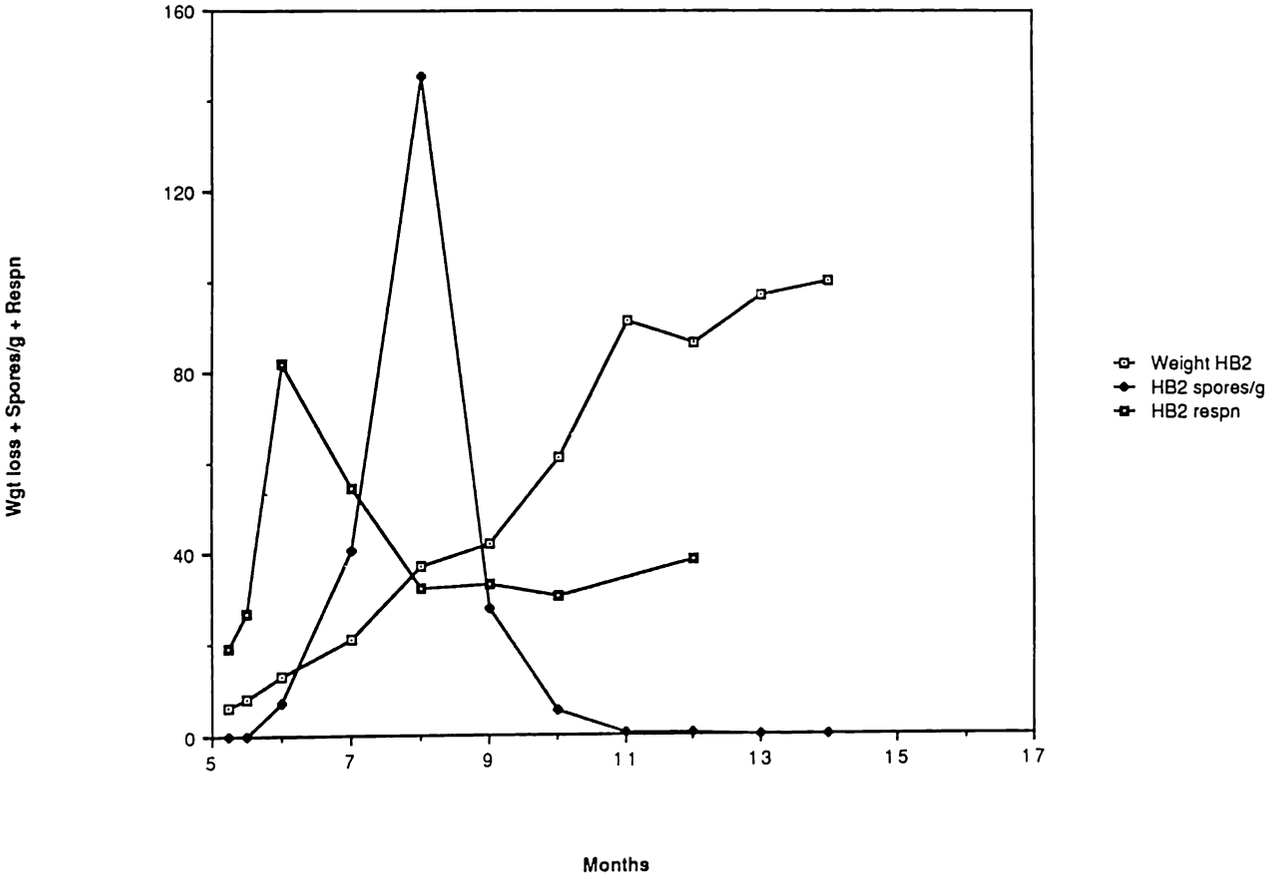

160
120
80
40
0
5
7
9
11
13
15
17
Wgt loss + Spores/g + Respn
Months
Weight HB2
HB2 spores/g
HB2 respn

Fig. 5.8: Weight loss (%), spore production (x 10^4 spores/g), and respiration rate (x 10^{-2} mg/g/hr) measured from kamahi leaves initially introduced into Reid's Ck in December and subsequently sampled at monthly intervals.

Fig. 5.9: Weight loss (%), spore production (x 10^4 spores/g), and respiration rate (x 0.25 x 10^{-2} mg/g/hr) measured from beech leaves initially introduced into Reid's Ck in December and subsequently sampled at monthly intervals.

Fig 5.8

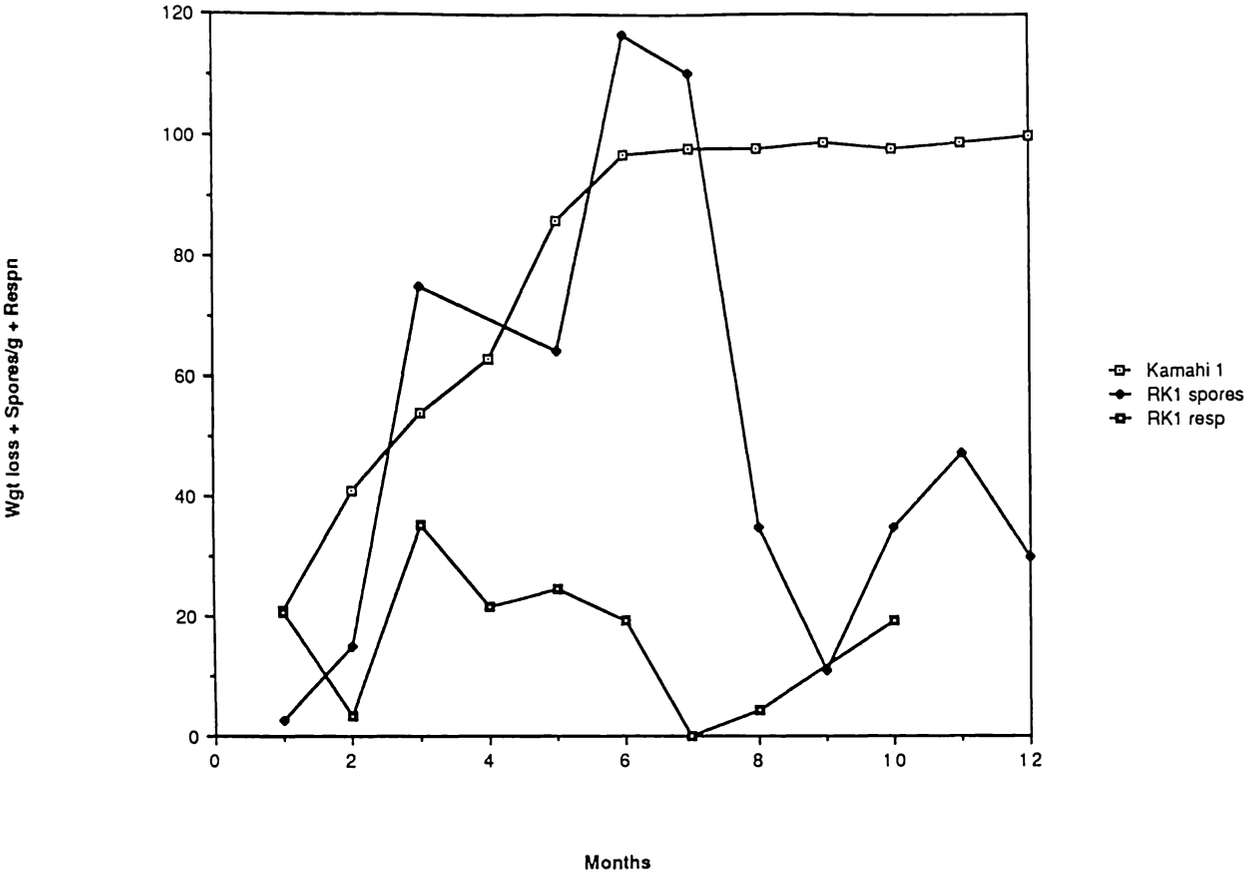

Wgt loss + Spores/g + Respn
120
100
80
60
40
20
0
0
2
4
6
8
10
12
Months
Kamahi 1
RK1 spores
RK1 resp

Fig 5.9

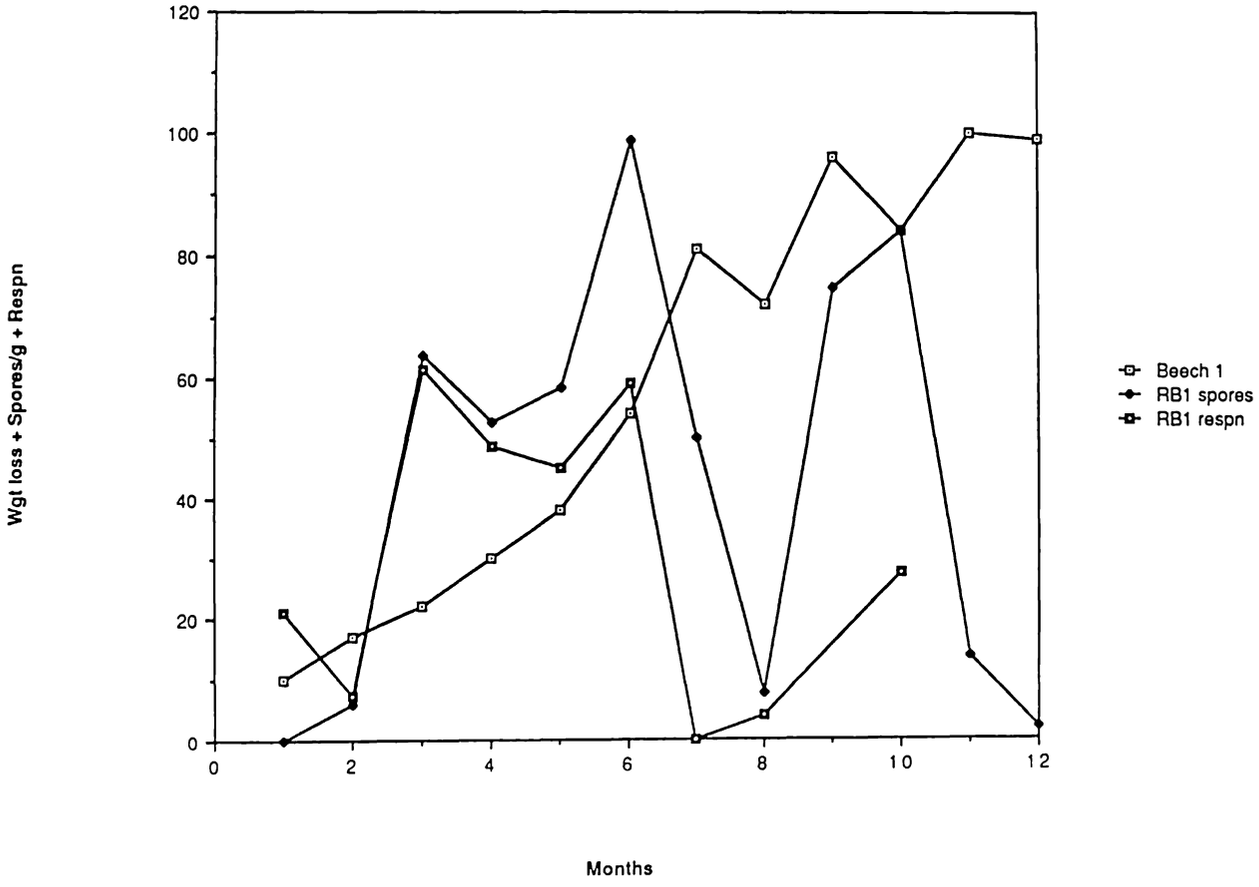

Wgt loss + Spores/g + Respn
120
100
80
60
40
20
0
0
2
4
6
8
10
12
Months
Beech 1
RB1 spores
RB1 respn

Fig. 5.10: Weight loss$_{(\%)}$, spore production (x 10^4 spores/g), and respiration rate (x 10^{-3} mg/g/hr) measured from kamahi leaves initially introduced into Reid's Ck in May and subsequently sampled at monthly intervals.

Fig. 5.11: Weight loss$_{(\%)}$, spore production (x 10^4 spores/g), and respiration rate (x 0.25 x 10^{-2} mg/g/hr) measured from beech leaves initially introduced into Reid's Ck in May and subsequently sampled at monthly intervals.

Fig 5.10

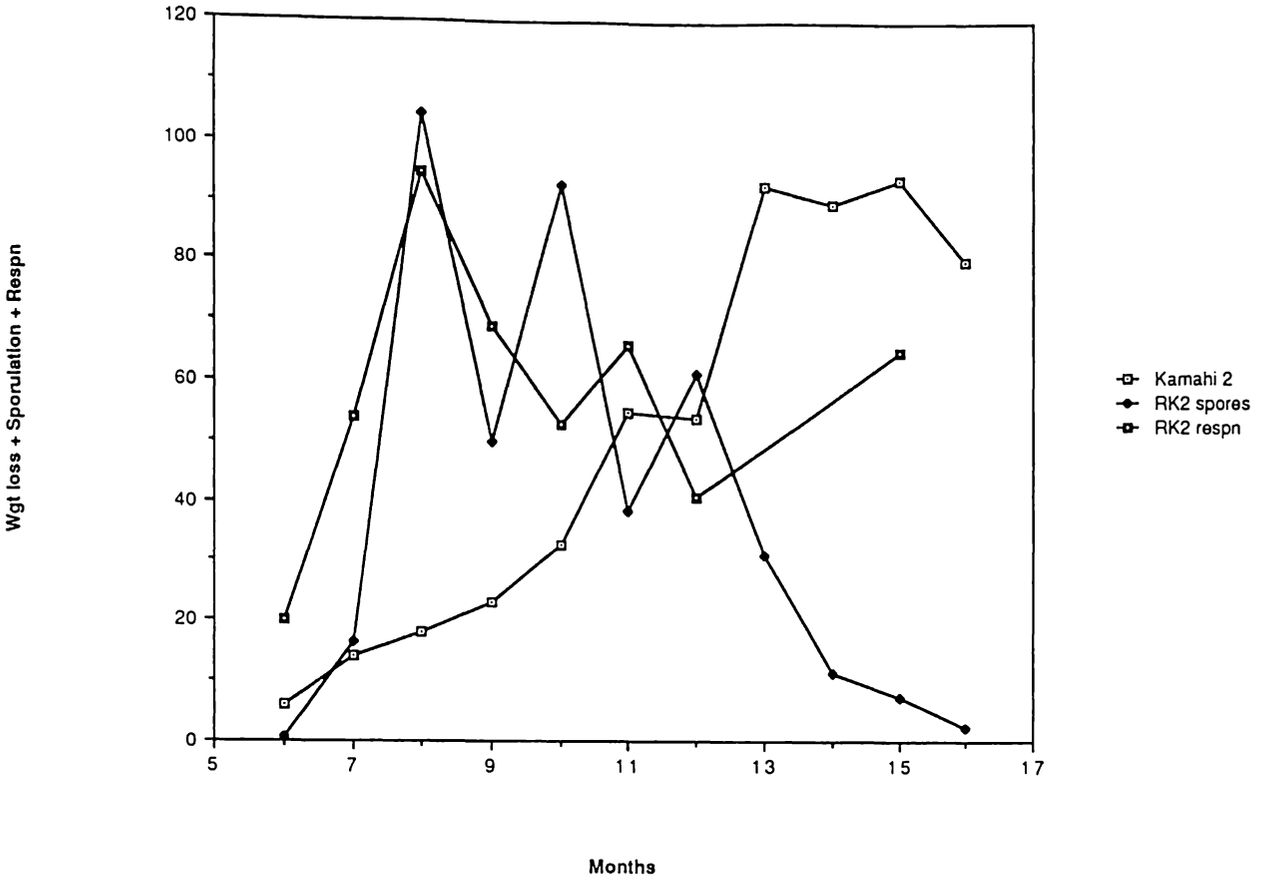
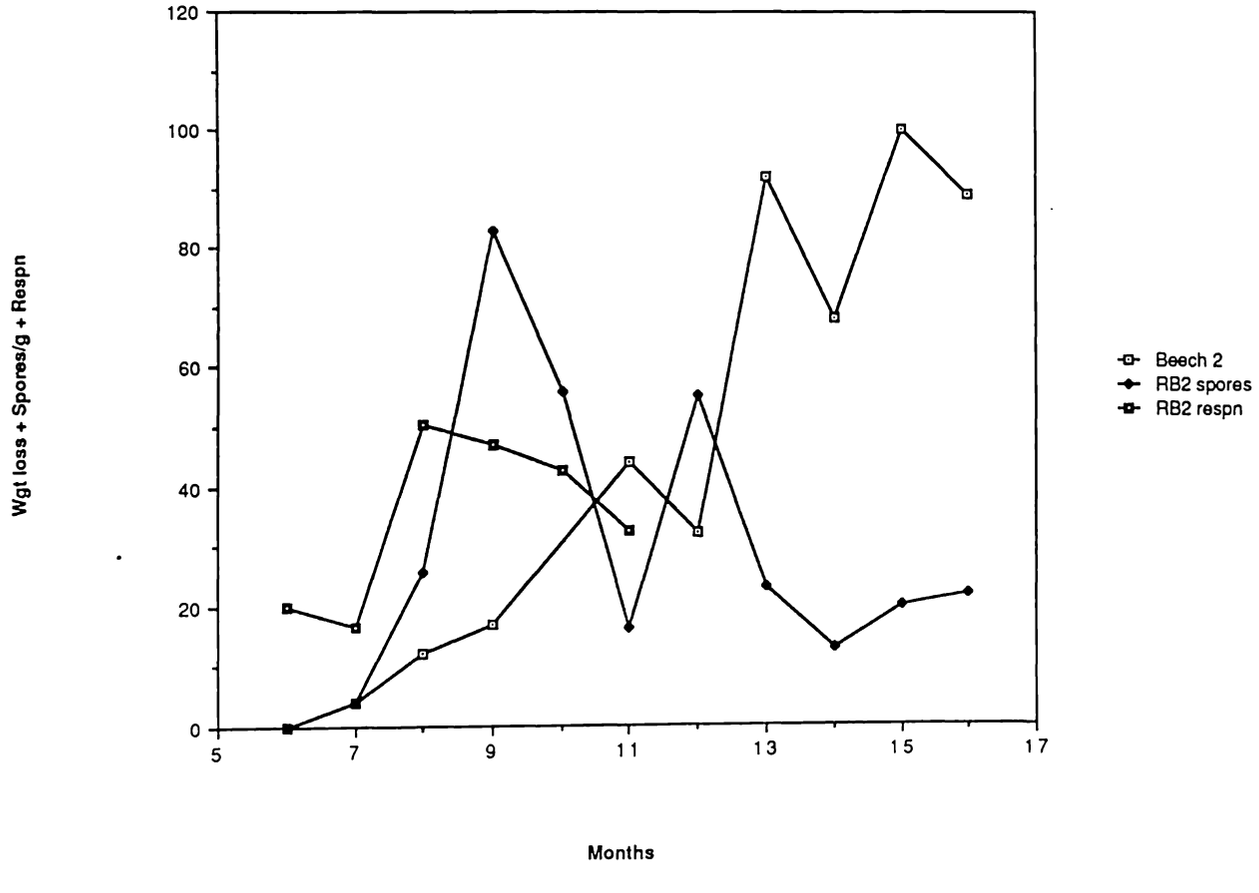


Fig 5.11



cases. Aquatic hyphomycetes might be expected to have the greatest direct impact on decay rates during this time. However, the peak in spore production (Figs 5.4 -5.11) occurred slightly prior to the second peak in decay rate (Figs 5.2 and 5.3) in general. This suggests aquatic hyphomycetes were less important to weight loss through direct consumption than, perhaps, as preconditioning agents for other sources of weight loss.

Spore production and respiration, after the normally high initial respiration of the first month, show a parallel initial increase in many cases. On second run kamahi (in both streams) they peaked and declined in parallel, and on second run beech in Reid's Ck there was only a month's lag between peaks in respiration and sporulation. However, in several cases (first run kamahi in both streams and first run beech in Reid's Ck) respiration declined while spore production was still high (coinciding with winter temperatures). In second run leaves (particularly in Hakarimata, where more respiration measurements were made late in decay), respiration did not decline as greatly as sporulation late in decay, in summer. Obviously, there was not a uniformly close correspondence between respiration and spore production from kamahi and beech, suggesting that other microorganisms may contribute quite significantly to respiration, and therefore leaf decay, particularly in summer.

The pattern of increase, peak, and decline in spore production from kamahi and beech (Figs 5.4 -5.11) was closely similar to that of invertebrate biomass (Tables 5.2 and 5.3), with the peak in invertebrate biomass coinciding with that in sporulation, or occurring shortly after it. Both declined at about the same time, or invertebrate biomass a little later. This suggests there may have been a relationship between aquatic hyphomycetes and

invertebrates and that part of the higher decay rates occurring after spore production declined may have been due to invertebrate consumption.

It is important to remember that while spore production relates directly to biomass, the relationship varies between species, and that the species composition could vary between leaf types, seasons and streams. For this reason biomass estimates may be more useful than sporulation figures in determining the pattern of aquatic hyphomycete abundance and therefore potential impact on leaf decay. Biomass figures are given in section 5.1.5.

5.1.4d Differences in spore production between seasons and streams.

Some differences between seasons and streams were seen in the figures for spore production and number of species. In Hakarimata 24 and 25 distinguishable species were found on kamahi in the first and second runs respectivly, compared to 32 in the first run and 30 in the second in Reid's Ck. In Hakarimata 19 and 22 species were found on beech in the first and second runs respectively, compared to 29 in the first run and 26 in the second in Reid's Ck. Thus there were consistently more species recorded from Reid's Ck, but no seasonal difference was apparent.

Differences between seasons and streams were also expected from the spore production from kamahi and beech (Table 5.6). Peak spore numbers in Hakarimata and Reid's Ck during the first run (summer) were closely similar. In the second run (winter), however, there were markedly greater numbers compared with the first run in Hakarimata but not in Reid's Ck. Spore production figures recorded from beech show similar diffences between runs to those from kamahi, with greater numbers in the second (winter) compared with the first run (summmer) in Hakarimata, but slightly less in the second

compared with the first run in Reid's Ck. The differences were not statistically significant (t test), but as they followed the same pattern on kamahi and beech they were probably real. The cause of differences in total spore numbers between seasons (runs) and streams may relate to the species composition, that is to whether the dominant species present are heavy sporulators (have low biomass/spore values) or not. For such differences (or similarities) to have ecological importance they should also be apparent from biomass figures (5.1.5).

5.1.5 Aquatic Hyphomycete Mycelial Content.

A number of conclusions were reached in the previous section regarding the pattern of aquatic hyphomycte abundance, as measured by spore production, during leaf decay, on different leaf types, in different seasons and in the two streams. It is important to remember that while spore production probably relates directly to biomass, the relationship varies between species, and that the species composition could vary between leaf types, seasons and streams. For this reason such differences (or similarities) would have ecological importance only if they were still apparent after the sporulation figures had been converted to mycelial lengths.

Using the mycelium:spore conversion factors determined in laboratory experiments (Table 7.1) the mycelial contents (m mycelium/g leaf material) of most of the numerically important species (according to spore production figures) could be calculated from the spore production figures given above. The *Lunulospora* spp. conversion factor was used for the remaining aquatic hyphomycetes on the assumption that this would not overestimate the mycelial lengths of most species. However, this was not necessarily the case for the

other conidial fungi recorded among the unidentified species, so where these spore types were present in significant numbers (for example, Unidentified sp. 4 on kamahi late in decay) the total mycelial content was not calculated. The results for kamahi and beech in both streams are shown in Figs 5.12 -5.15 (total mycelial content) and in appendices 4 -11 (individual species and total mycelial content).

Total mycelial content generally showed a similar pattern to total spore numbers: low at first, increasing to a peak at between 2 and 6 months (most commonly about 3 -4 months), then declining. Thus the general observations on the relationship between sporulation and weight loss, respiration, and invertebrate numbers also hold for mycelial content. That is, the peak in aquatic hyphomycete mycelial content was achieved prior to, or close to 50 % weight loss, slightly before the second peak in decay rate. The increase and peak in mycelial content was paralleled, after the first month, by the increase and peak in respiration. The decline in respiration generally preceded that in mycelial content in winter (first run), and more often followed it in summer (second run). The pattern of increase, peak, and decline in mycelial content was closely similar to that of invertebrate biomass.

Thus the relationships between weight loss, microbial respiration (and aquatic hyphomycete mycelial content), and invertebrate biomass fit in with the Petersen and Cummins (1974) scheme for the fate of leaves in streams. Their model envisaged initial rapid weight loss due to leaching being followed by microbial colonisation and conditioning, which in turn prepared the leaves for invertebrate consumption and physical wearing. All these processes appeared to occur in the present work, and their order and relative importance was reflected in the changes in decay rate Fig

Fig. 5.12: Aquatic hyphomycete mycelial content (m mycelium/g) estimated from spore production from kamahi leaves in Hakarimata (first run leaves (1) introduced into the stream in December 1982 = 0 months, and second run leaves (2) in May 1983 = 5 months), measured at monthly intervals.

Fig. 5.13: Aquatic hyphomycete mycelial content (m mycelium/g) estimated from spore production from beech leaves in Hakarimata (first run leaves (1) introduced into the stream in December 1982 = 0 months, and second run leaves (2) in May 1983 = 5 months), measured at monthly intervals.

Fig 5.12

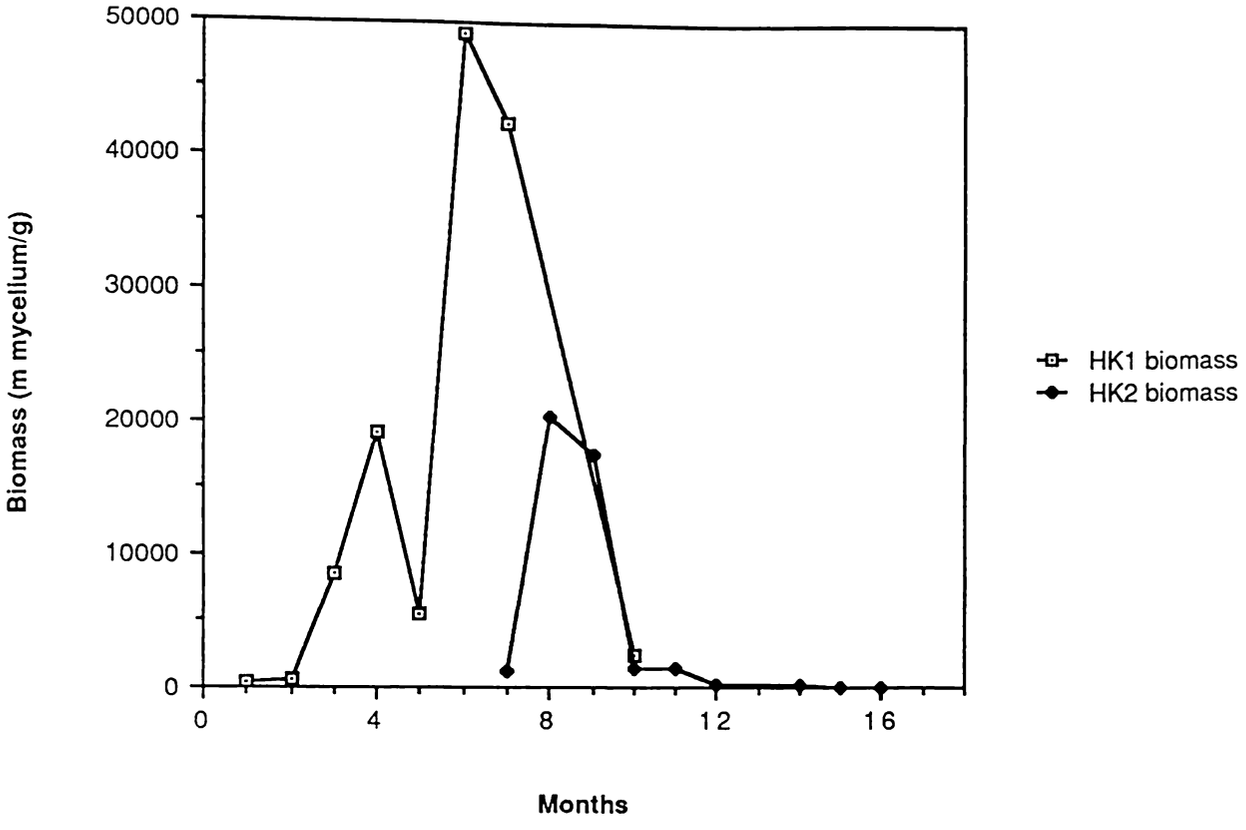


Fig 5.13

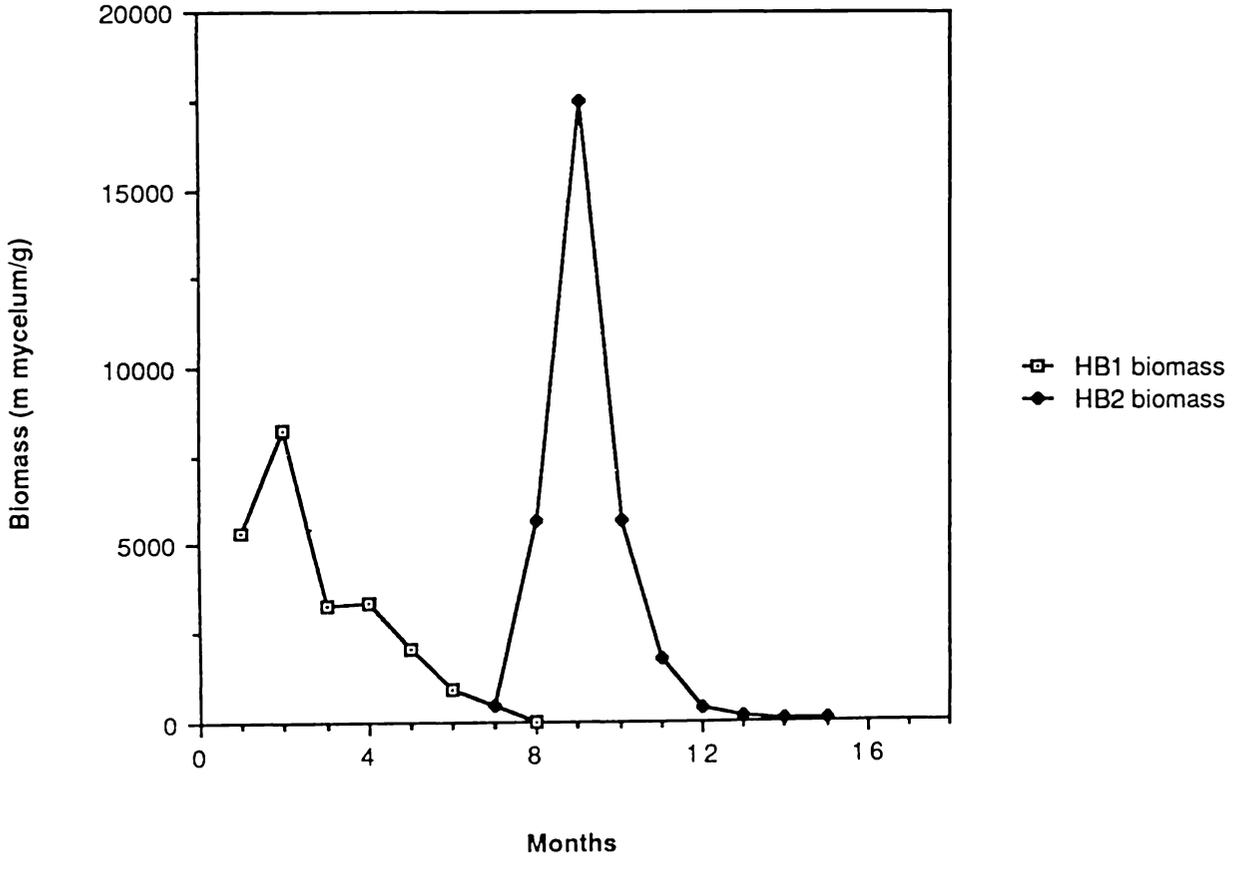


Fig. 5.14: Aquatic hyphomycete mycelial content (m mycelium/g) estimated from spore production from kamahi leaves in Reid's Ck (first run leaves (1) introduced into the stream in December 1982 = 0 months, and second run leaves (2) in May 1983 = 5 months), measured at monthly intervals. (Note 4 months/April value missing.)

Fig. 5.15: Aquatic hyphomycete mycelial content (m mycelium/g) estimated from spore production from beech leaves in Reid's Ck (first run leaves (1) introduced into the stream in December 1982 = 0 months, and second run leaves (2) in May 1983 = 5 months), measured at monthly intervals.

Fig 5.14

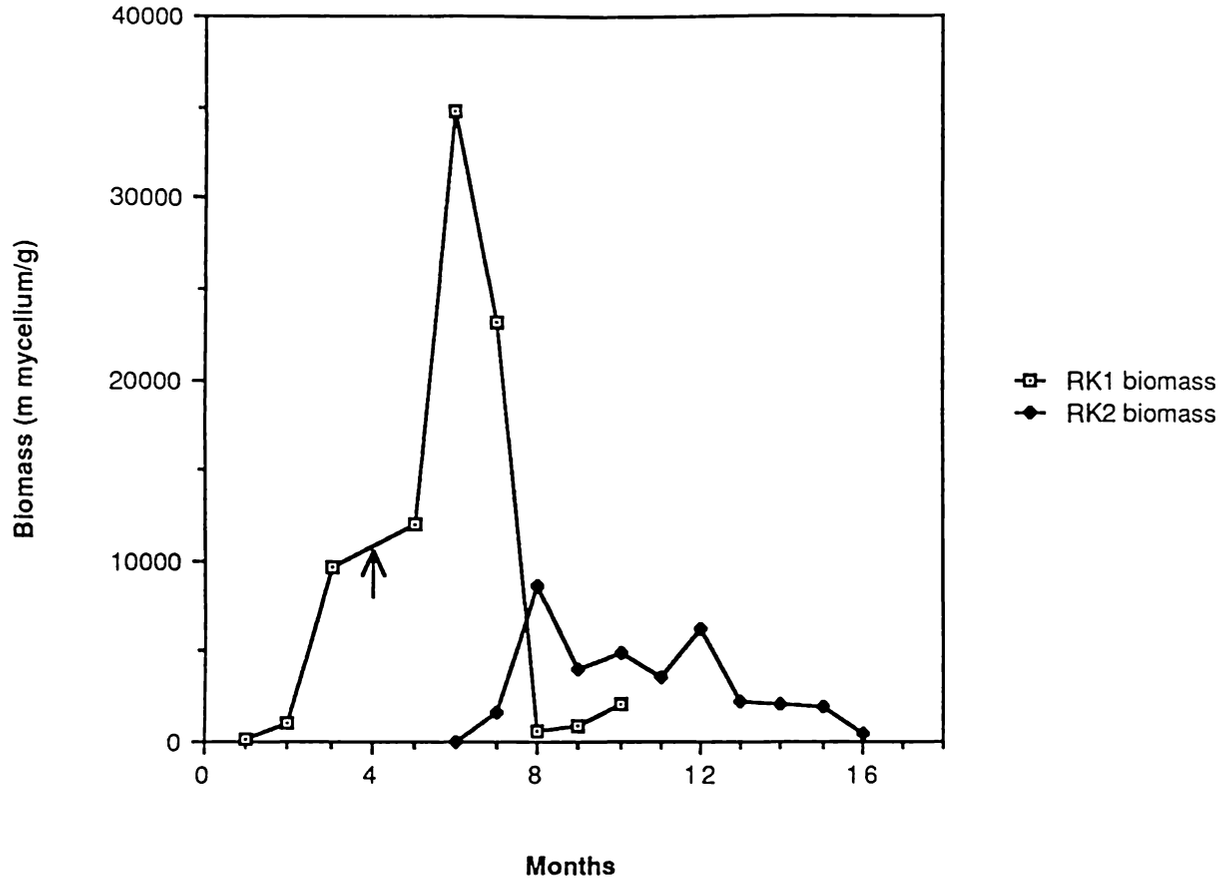
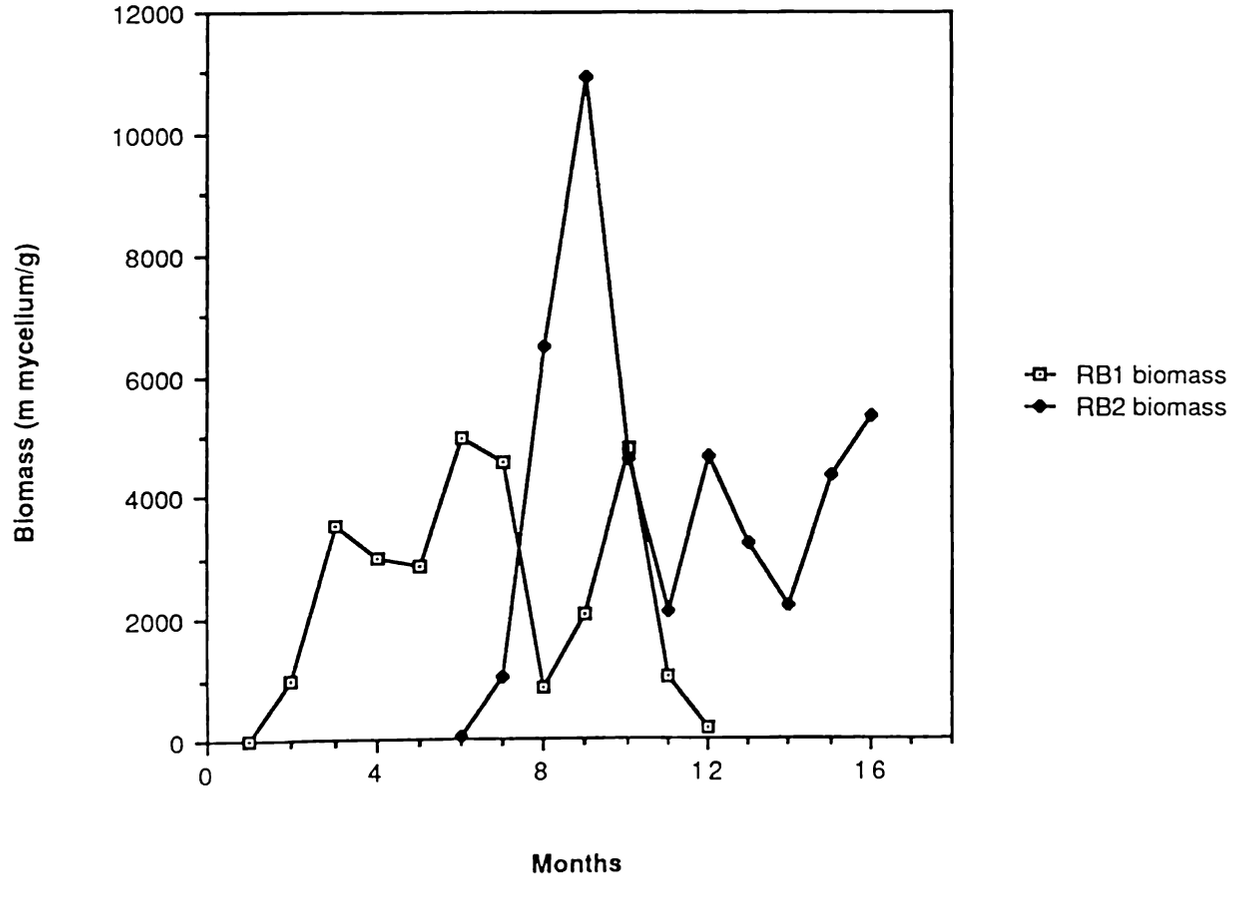


Fig 5.15



throughout decay (Figs 5.2 and 5.3). This is discussed further in section 8.1.3.

The peak and average mycelial content of kamahi leaves were markedly different from those of beech only in the first run of both streams, when the peak also appeared later on kamahi compared with beech. This coincided with a difference in decay pattern (interveinal tissue being lost more rapidly than the veins in kamahi but less so in beech) which was not as conspicuous in the second run. Thus there is no significant difference between the two leaf types in suitability for aquatic hyphomycetes when submerged in winter (second run), while in summer-introduced (first run) leaves the difference is certainly not of the scale expected from Davis and Winterbourn's (1977) work with mountain beech leaves.

There was a marked difference between the peak mycelial content of first and second run kamahi in both streams, but all four peak values occurred in June -September. The peak mycelial content of first and second run beech showed the reverse pattern, with the greatest values occuring in the second run in both streams. This corresponded to September -October. One of the smaller first run peaks also occurred in winter (May), but the other was in February. Thus there was some evidence for a seasonal pattern of higher mycelial content being recorded in winter and early spring, as suggested by Aimer (1982).

The maximum figures for mycelial content calculated from Hakarimata were consistently higher than those for Reid's Ck, following the same pattern as the sporulation results. However, the difference was not always very great, and the average mycelial content (Tables 5.7) were no different from each other, suggesting there was no difference in aquatic hyphomycete productivity between the streams, only in the length of time over which the productivity

was spread.

Some of the differences in aquatic hyphomycete abundance between leaf types, during decay, and between seasons and streams demonstrated in this section might relate to the preferences of individual species. The species present and their population dynamics in response to leaf decay, leaf type, season, and stream type are given in the next section.

5.1.6 Species Ecology of the Aquatic Hyphomycete Flora.

Some 48 spore types were distinguished from the leaves in the leaf bag experiment, but only 18 of them comprised 10 % or more of the average spore production from one leaf type or another (Table 5.7/7a). "*Lunulospora* spp." was generally the dominant spore type counted. It consisted largely of spores belonging to *L. cymbiformis*, with some spores possibly belonging to *L. curvula*. The two species were not regularly distinguished as the spores overlap in size and shape. The frequency of each species is discussed in 5.1.8 (random leaf collections).

The sporulation data for individual species on kamahi and beech provides information on: variation in abundance during leaf decay, i.e. successional patterns; variation between leaf types, i.e. substrate preferences; variation between runs, i.e. seasonal patterns; and variations between the two streams, i.e. ecological preferences. These are presented separately in the following four subsections

5.1.6a Successional Patterns.

Previous studies have differed as to whether a successsional pattern occurs among aquatic hyphomycetes during leaf decay. In the present work clear evidence was found of a successional pattern occurring during decay of kamahi and beech leaves, through the use of two overlapping experimental periods to allow separation of seasonal and successional phenomena.

The month by month spore production by species contributing more than 5% of the total spore numbers on more than one occasion on kamahi and beech is shown in Figs. 5.16 -5.19, as spores/g and in Figs 5.20 -5.23 as % of total spore production. Spore production by all species increased from initially low levels to a peak then declined again. A number of dematiaceous spore types (not graphed), probably belonging to the resident phylloplane mycoflora, showed a different pattern, with relatively high numbers in the first week or two but falling rapidly to below detection levels by the end of the first month.

The graphs of % of total spore production (Figs 5.20 -5.23) are arranged to show a sequence of peaks during both runs, and indicate that changes in species composition occurred during leaf decay. These sequences in % resulted from the interaction between the actual numbers of spores of each species present. The sequential nature of the peaks in spore numbers was not as clear as that of %, with groups of species tending to reach a peak at about the same time. Thus there was a successional pattern apparent between groups of species (rather than individual species) with regard to their times of maximum abundance.

Most species, if they were abundant at all in a stream or season, showed the same decay-related temporal placement of the peak in abundance on both beech and kamahi, in both runs and in both ~~Fig~~

Fig. 5.16 -5.19: Spore production (spores/g) by the major species, arranged in a sequence according to how rapidly peak production was reached. Species given are: *A. acuminata*, *Alatospora* spp., *A. filiformis*, *A. longissima/crassa*, *A. tetracladia*, *C. aquatica*, *C. longibrachiata*, *F. penicillioides*, *F. eccentrica*, *H. lugdunensis*, *L. aquatica*, *Lunulospora* spp., *L. ovalis* sp. ined., *T. elegans*, *T. marchalianum*, *T. chaetocladium*,, Unidentified sigmoid/rod type 1(2), 1(3), 12, 16, and 18. First run leaves (1) were initially introduced into the stream in December 1982 (= 0 months), and second run leaves (2) in May 1983 (= 5 months). Both were sampled after 1 and 2 weeks and then at monthly intervals from Hakarimata and at monthly intervals from Reid's Ck..

Fig. 5.16 = Kamahi leaves in Hakarimata.

Fig. 5.17 = Kamahi leaves in Reid's Ck.

Fig. 5.18 = Beech leaves in Hakarimata.

Fig. 5.19 = Beech leaves in Reid's Ck.

Fig 5.16

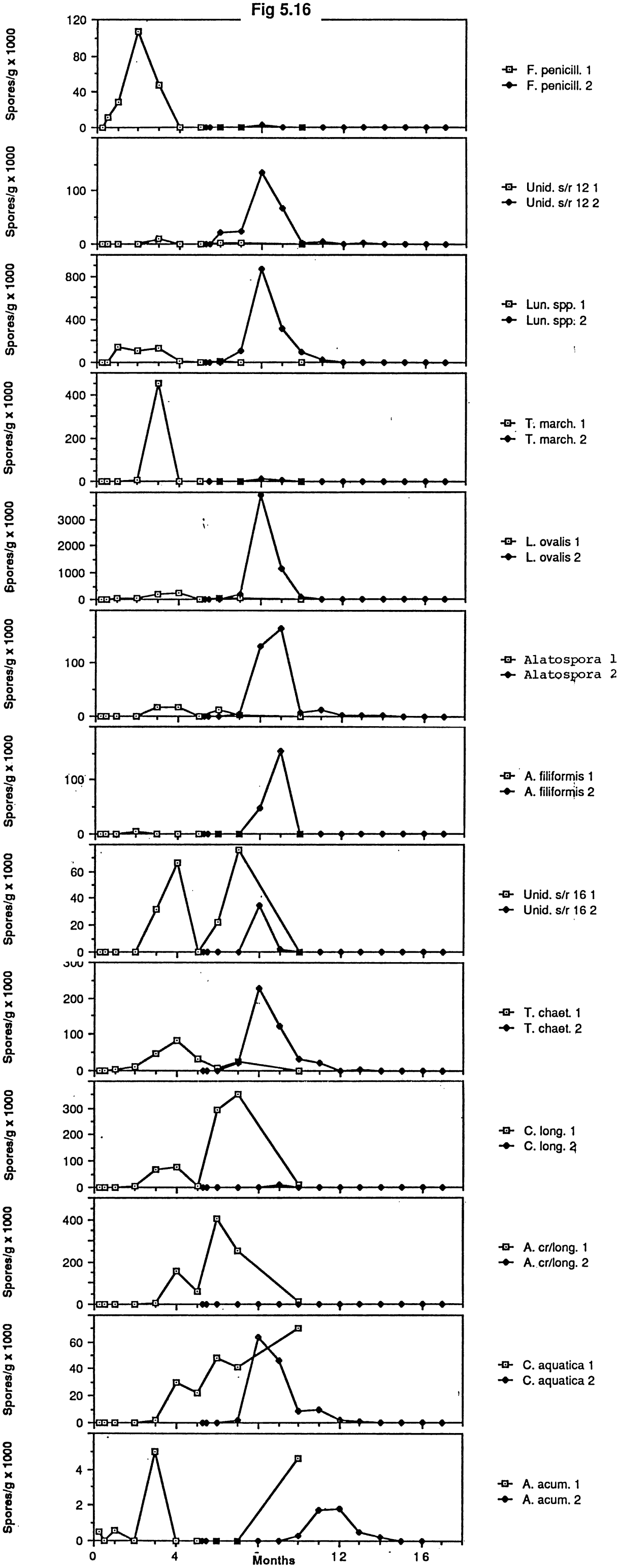

Spores/g x 1000
F. penicill. 1
F. penicill. 2
Unid. s/r 12 1
Unid. s/r 12 2
Lun. spp. 1
Lun. spp. 2
T. march. 1
T. march. 2
Spores/g x 1000
L. ovalis 1
L. ovalis 2
Alatospora 1
Alatospora 2
A. filiformis 1
A. filiformis 2
Unid. s/r 16 1
Unid. s/r 16 2
T. chaet. 1
T. chaet. 2
C. long. 1
C. long. 2
A. cr/long. 1
A. cr/long. 2
C. aquatica 1
C. aquatica 2
A. acum. 1
A. acum. 2
Months

Fig 5.17

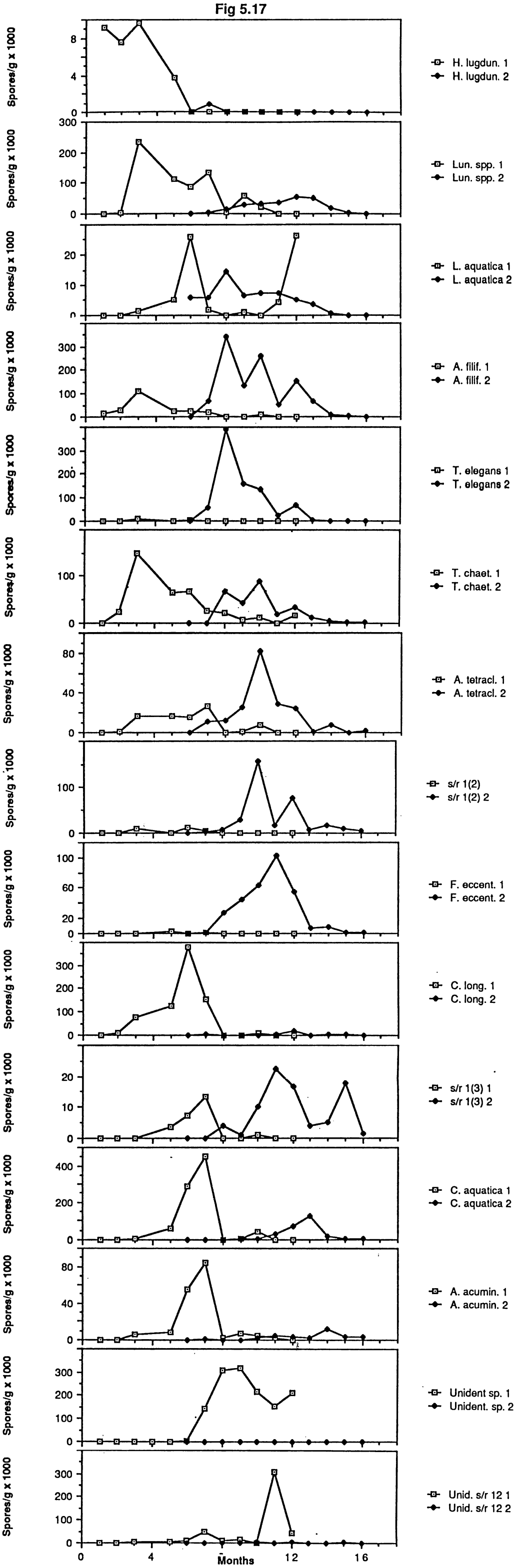

Spores/g x 1000
H. lugdun. 1
H. lugdun. 2
Lun. spp. 1
Lun. spp. 2
L. aquatica 1
L. aquatica 2
A. filif. 1
A. filif. 2
T. elegans 1
T. elegans 2
T. chaet. 1
T. chaet. 2
A. tetracl. 1
A. tetracl. 2
s/r 1(2)
s/r 1(2) 2
F. eccent. 1
F. eccent. 2
C. long. 1
C. long. 2
s/r 1(3) 1
s/r 1(3) 2
C. aquatica 1
C. aquatica 2
A. acumin. 1
A. acumin. 2
Unident sp. 1
Unident. sp. 2
Unid. s/r 12 1
Unid. s/r 12 2
Months
0
4
12
16

Fig 5.18

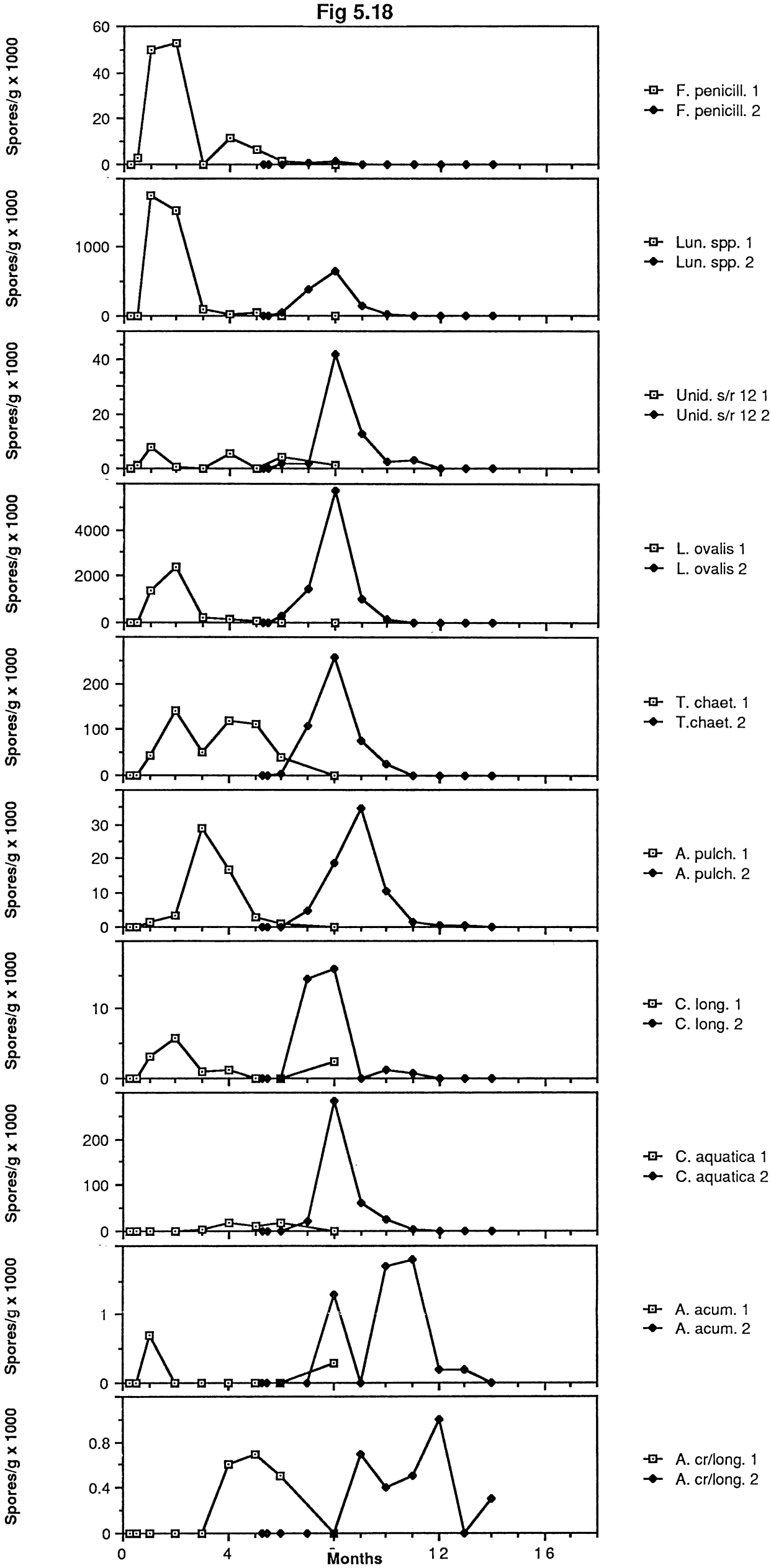

Spores/g x 1000
60
40
20
0
F. penicill. 1
F. penicill. 2
Spores/g x 1000
1000
0
Lun. spp. 1
Lun. spp. 2
Spores/g x 1000
40
20
0
Unid. s/r 12 1
Unid. s/r 12 2
Spores/g x 1000
4000
2000
0
L. ovalis 1
L. ovalis 2
Spores/g x 1000
200
100
0
T. chaet. 1
T.chaet. 2
Spores/g x 1000
30
20
10
0
A. pulch. 1
A. pulch. 2
Spores/g x 1000
10
0
C. long. 1
C. long. 2
Spores/g x 1000
200
100
0
C. aquatica 1
C. aquatica 2
Spores/g x 1000
1
0
A. acum. 1
A. acum. 2
Spores/g x 1000
0.8
0.4
0
A. cr/long. 1
A. cr/long. 2
0
4
8
12
16
Months

Fig 5.19

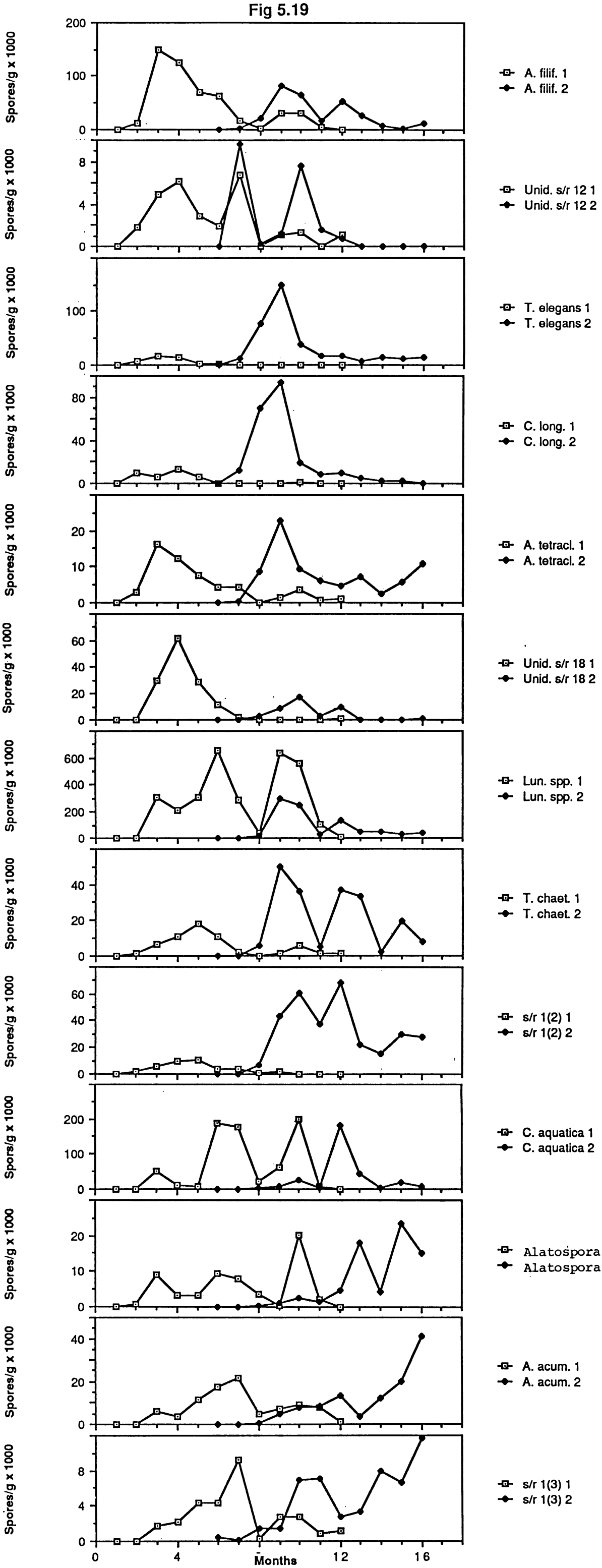

Spores/g x 1000
200
100
0
A. filif. 1
A. filif. 2
Spores/g x 1000
8
4
0
Unid. s/r 12 1
Unid. s/r 12 2
Spores/g x 1000
100
0
T. elegans 1
T. elegans 2
Spores/g x 1000
80
40
0
C. long. 1
C. long. 2
Spores/g x 1000
20
10
0
A. tetracl. 1
A. tetracl. 2
Spores/g x 1000
60
40
20
0
Unid. s/r 18 1
Unid. s/r 18 2
Spores/g x 1000
600
400
200
0
Lun. spp. 1
Lun. spp. 2
Spores/g x 1000
40
20
0
T. chaet. 1
T. chaet. 2
Spores/g x 1000
60
40
20
0
s/r 1(2) 1
s/r 1(2) 2
Spors/g x 1000
200
100
0
C. aquatica 1
C. aquatica 2
Spores/g x 1000
20
10
0
Alatospora 1
Alatospora 2
Spores/g x 1000
40
20
0
A. acum. 1
A. acum. 2
Spores/g x 1000
8
4
0
s/r 1(3) 1
s/r 1(3) 2
0
4
Months
12
16

Fig. 5.20 -5.23: Relative importance (% of total spore production) of the major species, arranged in a sequence according to how rapidly peak production was reached. Species given are: *A. acuminata*, *Alatospora* spp., *A. filiformis*, *A. longissima/crassa*, *A. tetracladia*, *C. aquatica*, *C. longibrachiata*, *F. penicillioides*, *F. eccentrica*, *H. lugdunensis*, *L. aquatica*, *Lunulospora* spp., *L. ovalis* sp. ined., *T. elegans*, *T. marchalianum*, *T. chaetocladium*,, Unidentified sigmoid/rod type 1(2), 1(3), 12, 16, and 18. First run leaves (1) were initially introduced into the stream in December 1982 (= 0 months), and second run leaves (2) in May 1983 (= 5 months). Both were sampled after 1 and 2 weeks and then at monthly intervals from Hakarimata and at monthly intervals from Reid's Ck..

Fig. 5.20 = Kamahi leaves in Hakarimata.

Fig. 5.21 = Kamahi leaves in Reid's Ck.

Fig. 5.22 = Beech leaves in Hakarimata.

Fig. 5.23 = Beech leaves in Reid's Ck.

Fig 5.20

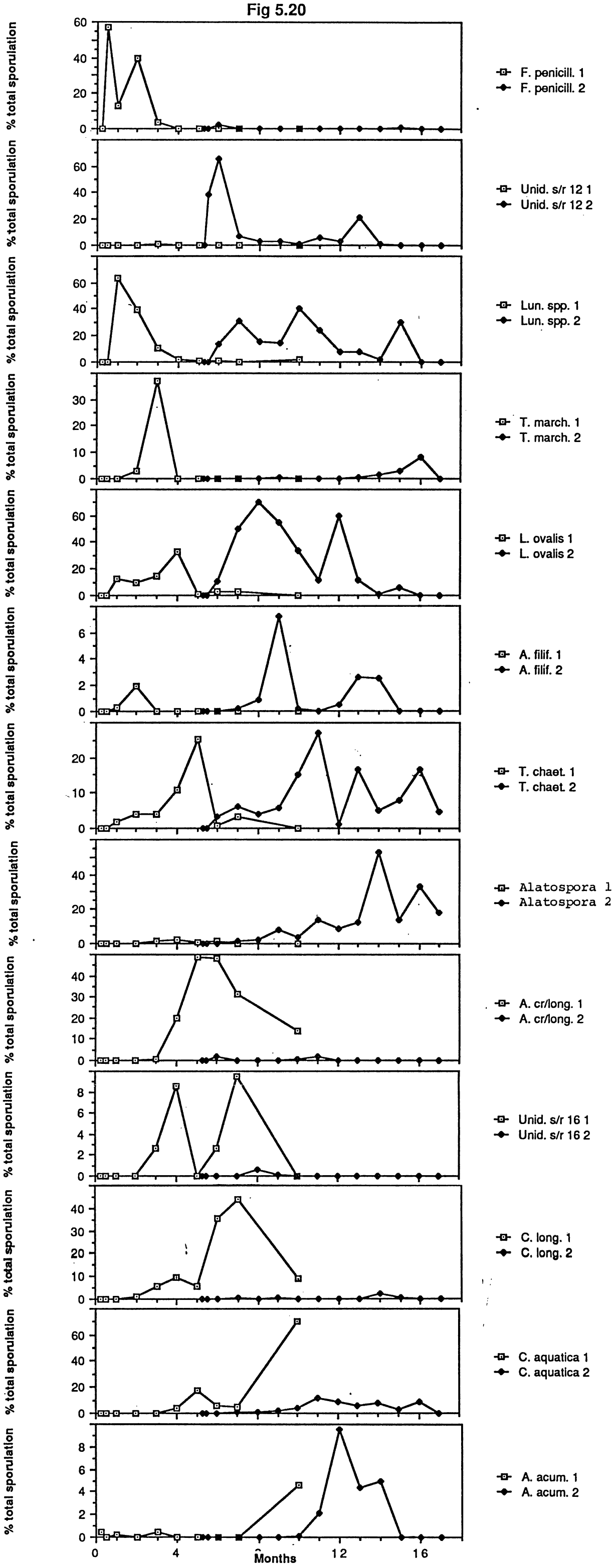

% total sporulation
F. penicill. 1
F. penicill. 2
Unid. s/r 12 1
Unid. s/r 12 2
Lun. spp. 1
Lun. spp. 2
T. march. 1
T. march. 2
L. ovalis 1
L. ovalis 2
A. filif. 1
A. filif. 2
T. chaet. 1
T. chaet. 2
Alatospora 1
Alatospora 2
A. cr/long. 1
A. cr/long. 2
Unid. s/r 16 1
Unid. s/r 16 2
C. long. 1
C. long. 2
C. aquatica 1
C. aquatica 2
A. acum. 1
A. acum. 2
0
4
8
12
16
Months

Fig 5.21

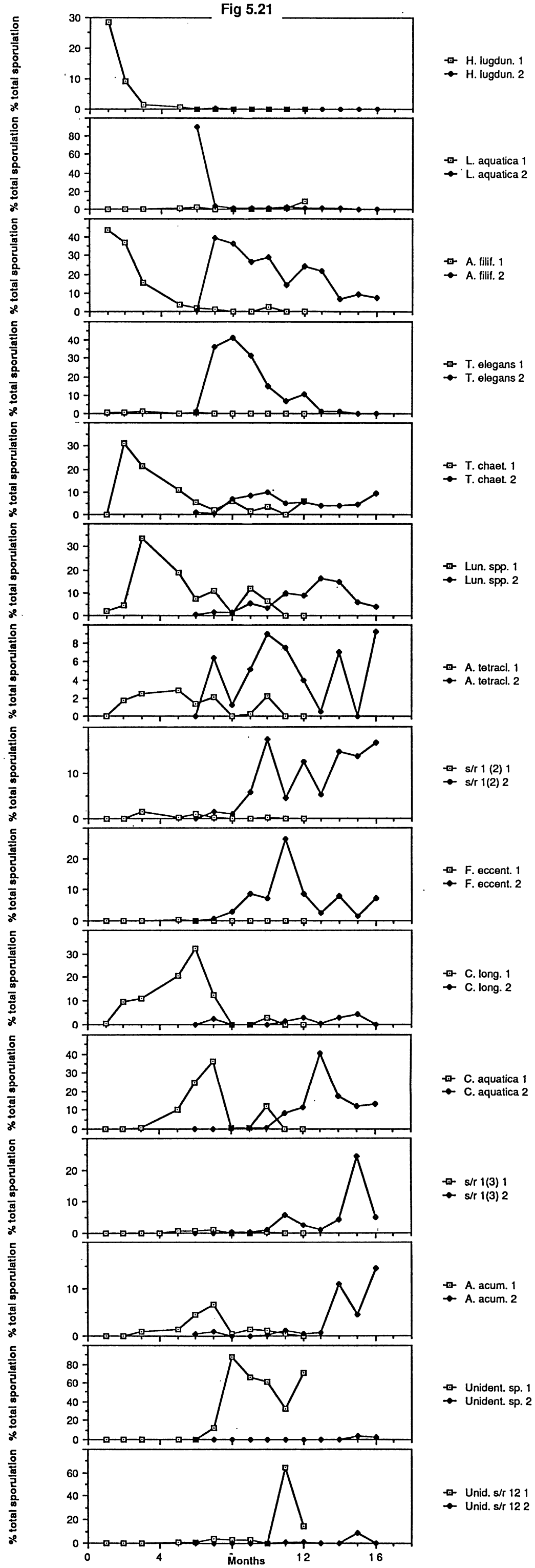

% total sporulation
H. lugdun. 1
H. lugdun. 2
L. aquatica 1
L. aquatica 2
A. filif. 1
A. filif. 2
T. elegans 1
T. elegans 2
T. chaet. 1
T. chaet. 2
Lun. spp. 1
Lun. spp. 2
A. tetracl. 1
A. tetracl. 2
s/r 1 (2) 1
s/r 1(2) 2
F. eccent. 1
F. eccent. 2
C. long. 1
C. long. 2
C. aquatica 1
C. aquatica 2
s/r 1(3) 1
s/r 1(3) 2
A. acum. 1
A. acum. 2
Unident. sp. 1
Unident. sp. 2
Unid. s/r 12 1
Unid. s/r 12 2
0
4
Months
12
16

Fig 5.22

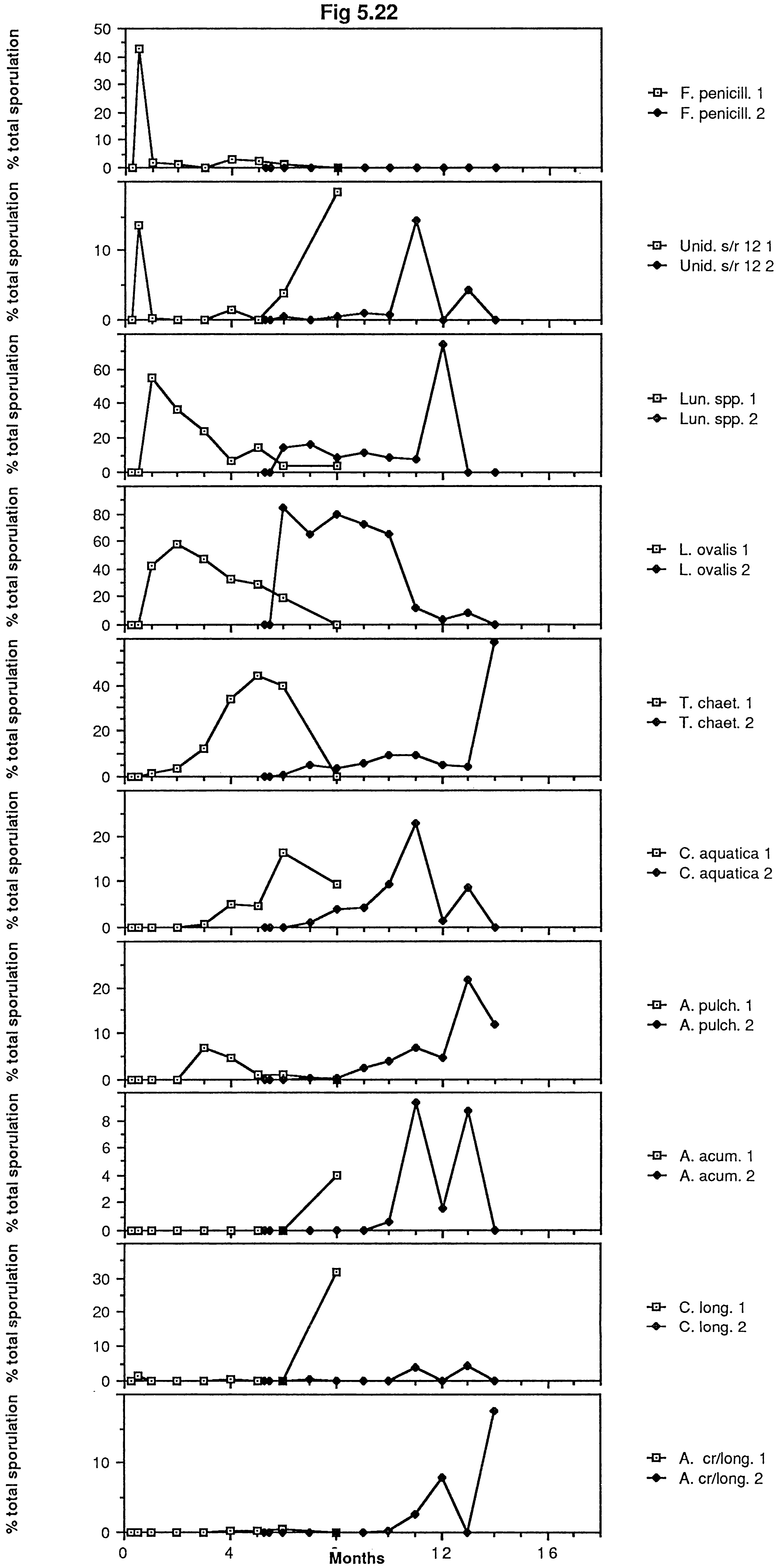
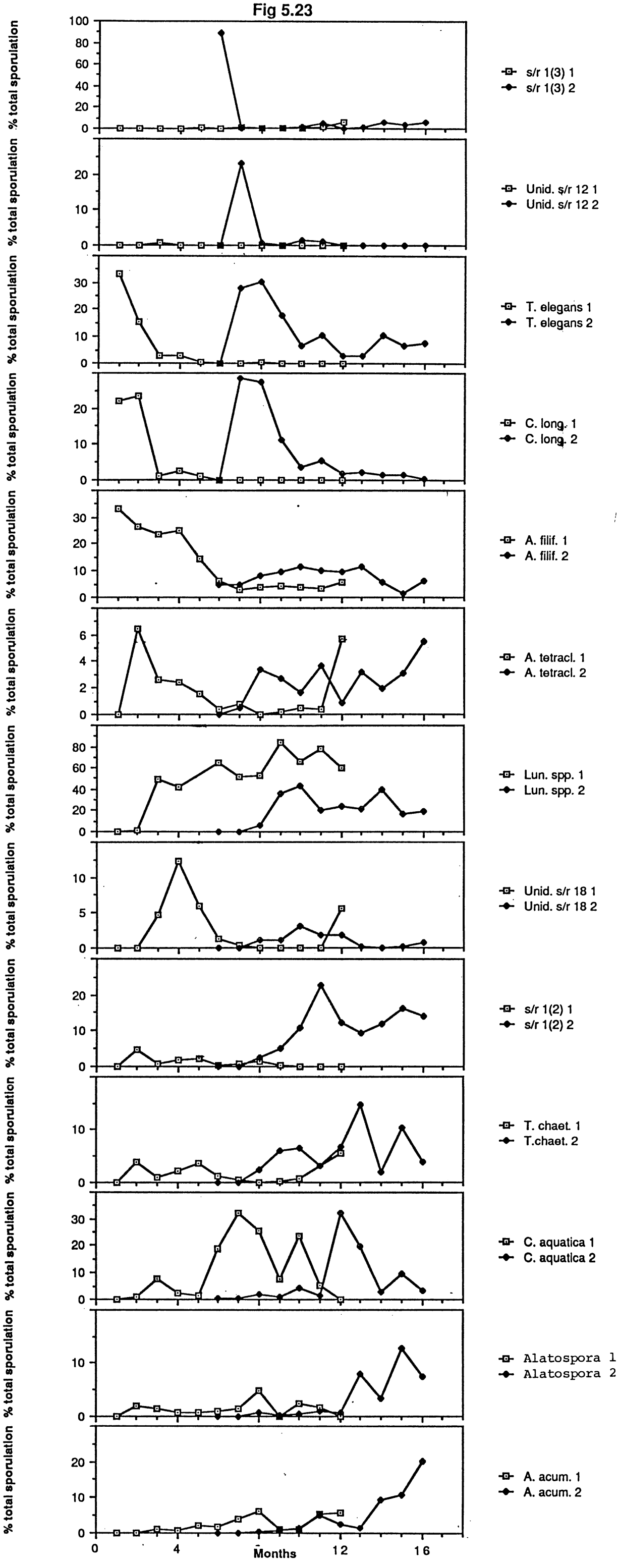

% total sporulation
Months
0
4
12
16
F. penicill. 1
F. penicill. 2
Unid. s/r 12 1
Unid. s/r 12 2
Lun. spp. 1
Lun. spp. 2
L. ovalis 1
L. ovalis 2
T. chaet. 1
T. chaet. 2
C. aquatica 1
C. aquatica 2
A. pulch. 1
A. pulch. 2
A. acum. 1
A. acum. 2
C. long. 1
C. long. 2
A. cr/long. 1
A. cr/long. 2

Fig 5.23


% total sporulation
s/r 1(3) 1
s/r 1(3) 2
Unid. s/r 12 1
Unid. s/r 12 2
T. elegans 1
T. elegans 2
C. long. 1
C. long. 2
A. filif. 1
A. filif. 2
A. tetracl. 1
A. tetracl. 2
Lun. spp. 1
Lun. spp. 2
Unid. s/r 18 1
Unid. s/r 18 2
s/r 1(2) 1
s/r 1(2) 2
T. chaet. 1
T.chaet. 2
C. aquatica 1
C. aquatica 2
Alatospora 1
Alatospora 2
A. acum. 1
A. acum. 2
0
4
12
16
Months

streams. Only two species differed. *C. longibrachiata* showed abundance patterns that varied between leaf types in both streams. On kamahi it reached a peak in abundance later in decay at 6 -8 months, after 50 % weight loss, whereas on beech it peaked at 1 -4 months, corresponding to 10 -20 % weight loss. Unidentified s/r type 12 also differed from the other species, having no consistent pattern and peaking in early, mid, and late decay on different leaf types or runs. The remaining species can be divided into three groups according to the time at which they were most abundant or important, with relative consistency.

EARLY DECAY SPECIES.

F. penicillioides and *H. lugdunensis* were the most rapid colonizers, achieving greatest relative importance (% of total sporulation) in the first month or so, although their peak in spore production usually occurred about the same time as a large number of slightly slower colonizers, generally prior to or close to 50 % weight loss.

MID DECAY SPECIES.

A. filiformis, *A. pulchella*, *A. tetracladia*, *L. ovalis* sp. ined., *Lunulospora* spp., *T. chaetocladium*, *T. elegans*, *T. marchalianum*, and probably Unidentified s/r type 1(2) peaked in importance after the most rapid colonizers. They usually reached a peak in spore production before or at 50 % weight loss (in the first 4 (Hakarimata) or 5 to 6 (Reid's Ck) months after immersion). Most declined to insignificant levels slightly before the end of decay. In Hakarimata this decline was clearly associated with a plateau in weight loss of 90 -99 %.

Within this group *T. chaetocladium*, *A. pulchella*, and Unidentified s/r type 1(2) showed a tendency to peak in numbers slightly later than the other species, while *A. filiformis* almost never did. *A. filiformis*, in the absence of either *F. penicillioides*, *H. lugdunensis* or Unidentified s/r type 12, appears to be the fastest colonizing species, peaking first in % importance.

LATE DECAY SPECIES.

This group consisted of *A. acuminata*, *A. longissima/crassa*, *C. aquatica*, Unidentified s/r type 1(3), and probably *F. eccentrica* and *L. aquatica* (abundant only on kamahi in Reid's Ck). Most appeared about the same time as the mid decay group, although *A. longissima/crassa* appeared slightly later fairly consistently. They peaked in numbers noticeably later, usually at 6 -8 months, or over 50 % decay, after the earlier groups had declined or started to decline to insignificant levels. They persisted in significant numbers until the end of the experiment, or close to it. In Reid's Ck, on kamahi, a non -aquatic hyphomycete spore type appeared as part of the succession even later in decay, appearing at 6 months and reaching a peak at 9 months.

The use of mycelial lengths rather than sporulation figures would not alter the observed sequence, but would affect the relative importance of each stage to total aquatic hyphomycete biomass during decay. Thus *F. penicillioides* and probably *H. lugdunensis*, the early decay species, had significantly lower mycelium:spore ratios than most mid and late decay species and their mycelium is unlikely

to be of any direct importance to leaf decay (weight loss) compared to the mid and late decay species.

As indicated in 5.1.5 (above) the time of peak biomass (maximum potential contribution to leaf decay) differed markedly between the first and second run of kamahi, resulting in an apparent seasonal pattern. The pattern was much less clear on beech. The peak mycelial content was comprised largely of late decay species on first-run kamahi but of mid decay species in second-run kamahi and beech. Thus although the peaks in mycelial content on first- and second-run kamahi occurred in the same season they involved different species, and were not simply a reflection of the seasonal preferences of all, or even of the dominant aquatic hyphomycetes in the streams. The abundance of late-decay species on first-run kamahi compared to second-run kamahi or beech may be better explained by the difference in decay pattern. Thus a pattern of relatively rapid loss of interveinal tissue followed by a stage as pale skeletal leaves favours the development of an abundant late decay flora more than a decay pattern which results in dark, poorly skeletonised terminal stages. However, the fact that the mycelial content of the mid-decay species on beech was greater in the second run (winter) than the first means that a seasonal preference, by either aquatic hyphomycetes as a whole (perhaps due to competition from other microorganisms, as suggested by Aimer, 1982), or by this mid-decay group at least, cannot be ruled out.

5.1.6b Substrate Preferences.

Most previous workers have found no firm evidence of substrate preferences by individual aquatic hyphomycetes compared to other species (Aimer, 1982; Bärlocher, 1980; Suberkropp and Klug, 1976). However, Bengtsson (1983), comparing alder and beech leaves,

Table 5.7: Spore production from the leaf types used in the leaf bag studies in Hakarimata and Reid's Ck presented as an average of all the samples in each leaf type/run/stream combination. The leaf types given here are kamahi (first and second runs, initially immersed in December and May), beech, fallen kamahi, and fallen beech. The average total spore production rates for all species (x 10^3 spores/g) is given in the first line, followed by the average percent of this derived from each species. The average mycelial content (m mycelium/g) calculated from the spore production from fresh kamahi and beech leaves is given in the last line.

	Kamahi				Beech				F. kamahi	F. beech
	Hak. 1	Hak. 2	Reid's 1	Reid's 2	Hak. 1	Hak. 2	Reid's 1	Reid's 2	Hak. 2	Reid's 2
TOTAL	443.83	599.85	397.68	372.37	951.23	1025.84	421.37	287.74	854.97	54.88
A. acuminata	0.01	0.05	3.89	0.87	0.03	0.05	1.78	3.52	0.03	9.84
Alatospora sp.	1.09	3.93	0.66	0.39	0.63	0.64	1.19	2.25	0.57	0.11
A. crassa+longiss.	20.05*	0.09	0.31	0.06	0.02	0.03	0.02	0.01	0.02	0
A. filiformis	0.24	2.43	5.10	26.80	0.60	0.02	10.10	9.13	0.25	8.60
A. tetracladia		<0.01	2.04	4.81		<0.01	1.08	2.47	0.24	1.53
C. sphagnorum				0.01						
C. filicladia	0.02									
C. aquatica	4.82	1.57	19.55	6.69	0.60	3.51	14.26	9.25	0.10	7.11
C. longibrachiata	18.22	0.14	17.30	1.61	0.16	0.29	0.74	7.07		11.77
Culic. aquatica			0.16	0.01			<0.01	0.02		
D. aquatica			0.07	0.28			0.05	0.66	<0.01	
E. microaquatica	4.02	1.27			0.88	1.89			0.28	
Filosporella sp.			2.54	0.15			0.07	0.33		
F. penicillioides	4.40	0.05			1.46	0.02			0.29	
F. eccentrica		<0.01	0.08	7.66			0.14	1.06	0.51	0.18
Gyoerffyella sp.										0.91
H. stellatacula	0.10	0.01	5.22	0.28		<0.01	0.16	0.71		0.07
H. lugdunensis			0.69	0.02			<0.01	0.09		
L. uniinflata										0.07
L. aquatica	0.02		1.48	1.40			<0.01	0.01		
L. ovalis	12.10	63.58		0.01	48.45	76.57			59.74	
Lunulospora spp.	9.19	16.94	14.99	5.94	40.35	11.28	61.97	28.46	27.49	24.34
M. acerina	0.01									
M. clavata				0.01						
T. gracilis				0.02			<0.01			
T. elegans		0.01	0.35	20.65	0.02	<0.01	0.90	11.36	0.01	6.12

Table 5.7 cont.

	Kamahi				Beech				F. kamahi	F. beech
	Hak. 1	Hak. 2	Reid's 1	Reid's 2	Hak. 1	Hak. 2	Reid's 1	Reid's 2	Hak. 2	Reid's 2
T. marchalianum	10.40	0.02			0.01	0.01			<0.01	
T. setigerum			0.06							
T. aquatica			0.02							
T. chaetocladium	4.76	5.14	8.97	6.71	5.87	4.20	1.17	6.26	7.45	3.46
T. splendens		<0.01	0.04			0.01	0.03	0.02		
T. acuminatus	0.56	0.01	2.65	0.02		<0.01	0.17	0.04		0.33
T. myrti				<0.01	<0.01				0.06	
Unident. sp. 1	0.17		0.01						0.01	
Unident. sp. 2			0.59	0.01			<0.01	<0.01		
Unident. sp. 4			31.87	0.09			0.21	0.05		
Unident. s/r 1(2)		0.71	0.66	8.20	0.28	0.01	0.78	9.86	0.12	3.21
Unident. s/r 1(3)		0.01	0.58	2.00			0.59	1.58	0.59	2.77
Unident. s/r 2	0.01		0.12	<0.01		<0.01	<0.01		1.18	
Unident. s/r 4	0.07		0.04	0.06	0.18		0.02	0.01		
Unident. s/r 10			0.28			<0.01	0.02		0.03	
Unident. s/r 12	0.36	3.10	9.22	0.52	0.03	0.55	0.71	0.66	<0.01	1.06
Unident. s/r 14		0.01								
Unident. s/r 16	4.43	0.43			0.19	0.75			0.02	0.04
Unident. s/r 18	0.66		0.06	<0.01			2.68	1.37		11.26
Unident. s/r 22		0.05								
unident. tetrarad.	0.07	0.03	0.20	4.41	0.06	0.01	0.45	3.21	<0.01	6.60
unident. sigmoid	0.73	0.13	0.41	0.22	0.17	0.03	<0.01	0.50	0.18	0.36
unident. spores	1.89	0.09	1.35	0.09	0.14	0.12	0.71		0.83	0.22
TOTAL BIOMASS:	16056	4726	13494	3236	3325	3521	2397	4080		

* A. longissima dominant.

Table 5.7a (5.7 continued): Spore production from the leaf types used in the leaf bag study in the Hakarimata stream presented as an average of all the samples in each leaf type. The leaf types given here are hangehange, young and old kanono, and young and old kauri (all initially immersed in December), and tawa, nikau, ponga, and rimu (initially immersed in May). The average total spore production rates for all species (x 10^3 spores/g) is given in the first line, followed by the average percent of this derived from each species.

	Leaf Types								
	Hange.	Yg kanono	Old kano.	Yg kauri	Old kauri	Tawa	Nikau	Ponga	Rimu
TOTAL	19.83	18.06	141.09	417.66	558.91	290.79	97.44	43.87	183.71
A. acuminata		2.33	0.06	0.04		0.01		59.27	0.82
Alatospora sp.		0.28	0.82	0.38	0.04	21.27	0.31	8.66	29.83
A. crassa+longiss.		0.28	13.40	0.08	0.04	0.17	0.46	0.05	0.92
A. filiformis	26.07	2.49	0.20	6.53	1.34	0.08			0.20
A. tetracladia	0.15								
C. filicladia			0.16			3.80			
C. aquatica			16.46	0.06	0.07	23.06	0.26	5.01	0.24
C. longibrachiata	0.66	11.52	10.30	2.49	0.08	0.03			0.03
E. microaquatica		2.77	3.85	3.71	8.44	1.85	0.02		1.25
Filosporella sp.	0.35			0.03					
F. penicillioides	1.51	34.33	0.34	3.33	27.31	0.23	0.06		7.84
F. eccentrica			0.04						
H. stellatacula			0.18		0.01				
L. aquatica			0.64			0.03			0.05
L. ovalis	40.19	7.64	23.35	71.09	43.30	2.63	0.06	0.68	2.97
Lunulospora spp.	21.33	19.93	12.69	3.89	11.60	11.12	0.15	0.46	23.39
Mycocentrospora sp.				0.06	0.02				0.05
T. elegans							0.04	0.07	
T. marchalianum		5.43	3.27			14.56		0.05	2.40
T. setigerum	1.20		0.57						
T. chaetocladium	2.02	9.41	2.18	4.97	3.67	4.01	0.67	0.68	7.68
T. splendens				0.01					
T. acuminatus				0.04	0.01		0.02		
T. myrti	5.40	2.33							
Unident. sp. 1						2.61	0.02		
Unident. sp. 3					0.02	0.01		18.81	0.30
Unident. s/r 1(2)		0.28	0.50	0.13					0.76

Table 5.7a cont.

	Hange.	Yg kanono	Old kano.	Yg kauri	Old kauri	Tawa	Nikau	Ponga	Rimu
Unident. s/r 2	0.16			0.05					
Unident. s/r 4	0.35				0.45	0.04			
Unident. s/r 10		1.00		0.10		0.01			
Unident. s/r 12			0.25	1.79	1.02	8.06		0.23	20.77
Unident. s/r 16			0.43	1.19	0.15				
Unident. s/r 21			8.49				4.82		
Unident. s/r 22			0.04						
Unident. sigmoids	0.66		1.86	0.05	0.11	0.03			0.15
Unident. spores						6.23			0.82
Dermat. sigmoids						0.14		6.04	
Ascospores							93.08		

suggested that while many species occur on a wide variety of leaf types, some species are more "resource specific" than others. In the present work different leaf types were poorly or well colonised by groups of species, or aquatic hyphomycetes generally, but patterns of substrate preference by individual aquatic hyphomycete species were not apparent among the dominant species.

Kamahi was taken as the reference type and all leaf types compared to it, except fallen beech, which was compared to green beech. Kamahi and beech could be compared using the figures for average species composition, over the whole of decay (given in Table 5.7/7a as spores/g and % of total spore number). Differences in average species composition between leaf types sampled monthly (kamahi and beech) and leaf types which were not sampled evenly throughout decay could be due to substrate preferences, or to differences in the amount of sampling at each successional stage. These could be distinguished between by using the month by month spore production figures (Appendices 12, 13, and 14) to compare the floras of leaves after the same length of time in the stream, or after the same amount of weight loss. Some differences in species composition, mostly minor, occurred, and some evidence for the successional pattern outlined above was obtained on a wider range of leaf types. These are summarised below for each leaf type (from Table 5.7/7a and Appendices 12, 13, and 14).

BEECH: The only species whose abundance was conspicuously different on beech compared with kamahi were species which only occurred in one stream or run, or only achieved a high importance value (%) on one occasion, e.g. *A. crassa/longissima*, *T. marchalianum*, Unidentified sp. 4, and possibly *L. aquatica* and *T. acuminatus*. There were no grounds for attributing these differences to substrate

preferences alone, rather than random effects, or perhaps a combination of factors. Thus *T. marchalianum* (mid decay) and *A. crassa/longissima* (late decay) achieved prominence only on first run kamahi in Hakarimata. This corresponded to the only occasion on which kamahi leaves showed a relatively rapid, soft-rotting mid decay phase, which then resulted in a long late decay phase as largely white vein fragments. The same decay pattern never occurred in beech.

FALLEN KAMAHI AND BEECH: The same successional pattern occurred on senescent kamahi as green. That is, early species (*F. penicillioides*) reached a peak in numbers first. The mid decay species followed immediately, dominated by *L. ovalis* sp. ined., *Lunulospora* spp., and to a lesser extent *T. chaetocladium*. *Alatospora* spp. and *C. aquatica* were slowest in reaching peak numbers. The late decay species, as on second run kamahi, were not as abundant as on first run kamahi, and because of the decreased sampling frequency, were poorly sampled. Naturally senescent beech leaves showed a similar relationship to green beech. Thus green and senescent (fallen) leaves were closely similar with regard to their mycoflora as well as the total abundance of aquatic hyphomycetes (as shown in 5.1.4).

HANGEHANGE: Early- and mid-decay stages were not distinguishable, and the whole late-decay group of species was absent, suggesting decay was too rapid and truncated to allow their development.

KANONO: Late-decay species were low on young kanono, as on hangehange, along with a number of mid-decay species most common on kamahi, such as *Lunulospora* spp. and *L. ovalis* sp. ined. *F. penicillioides* appeared as a relatively rapid colonizer of young and old kanono, as on kamahi, but was not as clearly an early-decay species. The same mid- and late-decay species occurred on old kanono as on first run kamahi and showed similar relative abundances. Thus in both first run kamahi and old kanono a period of relatively rapid early decay during summer apparently limited the levels of mid decay species seen and was followed by a longer period of slower late decay during which a variety of late decay species occurred in abundance, coming to dominate the average total spore production (Table 5.7a). It is possible that seasonal effects during the late decay phase were also important. These will be considered in the next section (5.1.6c).

KAURI: The pattern of abundance of early, mid and late species on young kauri was little different from that on old kauri.

Although placed in the stream at the same time as first run kamahi, and colonised by the same aquatic hyphomycete species, in roughly the same order, each decay stage continued over a longer period, corresponding to the slower decay of kauri. For example, mid decay species reached a peak in the 8 month sample (30 % weight loss), at levels closely comparable to those recorded from kamahi after 3 months (42 -55 % weight loss). Late-decay species, however, were only seen occasionally and at low numbers (as a group) right to the end of the experiment, despite the kauri leaves reaching 90 % weight loss in the final, 17 month, sample. This may relate to the decay pattern in late decay. Kauri, like beech and second-run kamahi, rotted through dark terminal stages consisting of sparse

skeletons with fragments of lamina, compared with the pale, more finely skeletonized stages of first-run kamahi and of kanono.

NIKAU: Two samples were taken, after 6 and 12 months immersion. At 6 months 38 % weight loss had occurred, from which a mid-decay flora might be expected. In contrast, many of the most important mid-decay species are relatively low (% and numbers), particularly *L. ovalis* sp. ined. Two of the 5 most abundant species were late-decay species. The most abundant mid-decay species, *T. chaetocladium*, tended to be more important later in the mid-decay phase on kamahi, for example, being the dominant species on second run kamahi at 6 months. Thus the flora on nikau at 38 % weight loss approached a late decay flora, and was closer to that found on the 73 % decayed kamahi, sampled on the same date, than to 40 % decayed kamahi (2 -3 months). It is concluded that, perhaps because of the greater content of veins and other more resistant tissues in nikau, less weight loss was required to reduce it to an aquatic hyphomycete habitat similar in some crucial way to 70 % decayed kamahi.

Very few aquatic hyphomycetes remained at the 12 months sample (81 % weight loss), the mycoflora being apparently dominated by several species of ascomycetes (see chapter 3).

RIMU: By the 6 month sample 40 % weight loss had occurred, from which a mid-decay flora might be expected. However, *F. penicillioides*, an early-decay species, was more abundant than expected. The normally dominant mid-decay species, *L. ovalis* sp. ined., *Lunulospora* spp., and *T. chaetocladium*, were low, but levels of late-decay species were also low. The dominant aquatic hyphomycete on rimu, *Alatospora* spp., tended to be most common later

in the mid-decay phase of kamahi. Thus the flora on rimu at 6 months resembled that found on kamahi relatively late in mid decay, at 50 -70 % weight loss, compared with 40 % weight loss in the rimu. The only species which differed in abundance from this decay-determined flora, and that might therefore be modified by the substrate type, was *F. penicillioides*.

At 12 months (84 % weight loss) rimu, like nikau, bore a very reduced mycoflora dominated by species other than known aquatic hyphomycetes, in this case Unidentified s/r type 22 and Unidentified sp. 4.

PONGA: At 6 months 25 % weight loss had occurred, and an early- to mid-decay flora would be expected. However, late-decay species dominated the flora, in particular *A. acuminata*, along with *Alatospora* sp. (a mid- to late-decay species) and an unidentified dematiaceous species. Thus the flora coincided most closely with that found on kamahi at 9 months (93 % weight loss). This suggests ponga was a rather poor substrate for aquatic hyphomycetes and related fungi: after only 25 % weight loss it was equivalent to 93 %-decayed kamahi as a substrate for aquatic hyphomycetes.

At 12 months 59 % weight loss had occurred. Spore numbers were about the same, however, and the flora was still dominated by the same late decay species, together with Unidentified sp. 4.

TAWA: Weight loss had reached 37 % at the 6 month sample, suggesting a mid-decay fungal flora might be expected. This is the case. Although some of the mid-decay species normally dominant (*Lunulospora* spp., *L. ovalis*) were overshadowed by normally lesser

species, the spore production values were all within the range seen on kamahi at about the same amount of weight loss.

By 12 months there were little more than petioles remaining of the leaves. The flora was dominated by species other than aquatic hyphomycetes (mainly *C. filicladia*). The abundance of *C. filicladia* was conspicuously different from all other leaf types studied. However it seems unlikely that this one leaf type should have a substrate-specific species when no other leaf type did. In addition *C. filicladia* was not recorded from the random litter samples from Hakarimata, which were often dominated by tawa.

In summary, there was no firm evidence for substrate preferences between most of the leaf types (kamahi, beech -both green and senescent, kanono, kauri, rimu, and tawa). Late decay species tended to be less prominent on leaves with dark, poorly skeletonised terminal decay stages and on very fast-decaying leaf types. In contrast nikau and ponga had sparse aquatic hyphomycete floras that were dominated by late decay species at a stage when little weight loss had occurred. Evidence of a successional pattern appeared on old kanono and on kauri leaves (in addition to kamahi and beech).

5.1.6c Seasonal Patterns.

Some previous Northern Hemisphere workers have found quantitative differences in seasonal occurrence between species, either as spores in stream water or in their occurrence on leaf litter (Triska, 1970; Gonczöl, 1975; Webster et al, 1976; Suberkropp, 1984; Shearer and Webster, 1985b). The present work has found evidence that aquatic hyphomycetes generally were more

abundant in the leaf bag leaves in winter (5.1.5), but this was explained in part by a difference in the decay pattern in summer-introduced leaves favouring mid- or late-decay species (5.1.6a). However, clear seasonal differences were found between species in the present work and these are covered here.

Many of the most abundant species on both kamahi and beech showed higher peak and average abundances in either the first or the second run (visible as % in Table 5.7 and as spores/g in Figs 5.16 -5.19). The first and second runs coincided, during their first few months, with summer or winter respectively, when colonization was occurring together with much of the growth to peak levels of the early- and mid-decay species. The reverse coincidence appeared with regard to late-decay species: higher abundances in the first run represented a winter peak and vice versa.

In Hakarimata *F. penicillioides* and possibly *T. acuminata*, Unidentified sp.1, and Unidentified s/r type 16 were more abundant in summer. *Lunulospora* spp. were more abundant in summer on beech, but in winter on kamahi, although numbers may have been suppressed in the first run (summer) by the rapid initial decay rate. *A. filiformis*, *C. aquatica*, *C. longibrachiata*, *L. ovalis* sp. ined., and Unidentified s/r type 12 were more abundant in winter. *A. pulchella* and *A. longissima/crassa* followed the same pattern on kamahi but did not show any clear pattern on beech, while *T. marchalianum*, *T. chaetocladium*, and Unidentified s/r type 1(2) did not show a clear seasonal pattern on either substrate. *C. aquatica* appeared to be the most strongly seasonal species, being most abundant in winter independent of the stage of decay on kamahi in Hakarimata at least (Fig. 5.16).

In Reid's Ck *Lunulospora*, Unidentified s/r type 18, and *T. acuminata* were clearly more abundant in summer. *H. lugdunensis* showed a similar pattern on kamahi, but no clear pattern on beech. *C. longibrachiata* was more abundant in the first run on kamahi and in the second run on beech (corresponding to winter in each, due to a change in successional position). *A. filiformis* was more abundant in winter on kamahi, but summer on beech. *F. eccentrica*, *T. elegans*, and Unidentified s/r type 1(2) were more abundant in winter on both leaf types. *A. acuminata*, *A. tetracladia*, and *C. aquatica* showed a pattern of greater abundance in winter on kamahi, but no clear pattern on beech. *T. chaetocladium*, and *D. aquatica* also tended to be more abundant in winter, but the difference was not great. *A. longissima/crassa*, *L. aquatica*, Unidentified s/r type 1(3), and Unidentified s/r type 12 showed no clear pattern on either leaf types.

Clearly then, there was an interaction between season and stream for many species. Species which were more abundant in the first run (summer species) in Hakarimata were absent from Reid's Ck, e.g. *L. ovalis*, or more abundant in summer e.g. *F. penicillioides*, Unidentified s/r type 16, and *T. acuminata*. Species marginally or clearly more abundant in winter in Hakarimata tended to be without seasonal pattern or marginally more abundant in summer in Reids Ck e.g. *Alatospora* spp., *A. longissima/crassa*, *A. acuminata*, Unidentified s/r type 12, *C. aquatica*, and *A. filiformis*. Species which were more abundant in winter in Reid's Ck were very low or absent in Hakarimata e.g. *F. eccentrica* and *T. elegans*. Some species did not

follow these patterns, however, for example *T. chaetocladium* was a marginal winter species in both streams; *L. ovalis* sp. ined. was a winter species in Hakarimata but was absent from Reid's Ck; *H. lugdunensis* was apparently a summer species in Reid's Ck but was absent from Hakarimata; *A. tetracladia* and Unidentified s/r type 1(3) were present only in Reid's Ck but were only marginal winter species.

5.1.6d Differences Between Streams.

There were obvious differences in the species composition of Hakarimata and Reid's Ck as observed on the kamahi and beech leaf bag leaves (Table 5.7). *A. longissima/crassa*, *L. ovalis* sp. ined., *F. penicillioides*, and *T. marchalianum*, were present or important (5 % or more of total spore production) only in the Hakarimata stream. *A. tetracladia*, *F. eccentrica*, *H. lugdunensis*, *L. aquatica*, *T. elegans*, Unidentified s/r type 1(2) and possibly 1(3) were present or important only in Reid's Ck.

The two streams were also compared through a number of stream measurements and through collections of naturally occurring leaf litter (5.1.7 and 5.1.8).

5.1.7 Stream Measurements: Aquatic Hyphomycete Spore Concentration and Temperature.

Aquatic hyphomycete spore concentrations in the stream water and water temperature were measured at monthly intervals in Hakarimata and Reid's Ck during the period of the leaf bag experiment. The purpose of these measurements was to test for differences in species composition between the two streams and between seasons, to support the observations made from the leaf bag leaves, and to measure the sample to sample variation which could be expected in the stream survey. In addition, the one previous study of seasonal periodicity in a New Zealand stream (Aimer and Segedin, 1985a) followed spore numbers for only a single year. The present study aimed to confirm the observed pattern by testing for a similar seasonal fluctuation in total spore numbers. Temperature was recorded as the most obvious difference between the two streams, being responsible for the difference in riparian vegetation, and possibly playing a role in seasonal preferences of aquatic hyphomycetes.

Water temperatures recorded are given in Fig. 5.24 (maximum, current, and minimum), under the graphs of spore concentration in each stream (presented as spores/l and spores passing a given point per second for total spores). Temperatures in Reid's Ck differed from Hakarimata most notably in the lower end of the range, being colder in winter (0.5 -20 C compared with 5 -18 C).

Seasonal patterns in spore numbers were apparent, total spore numbers being higher in summer and lower in winter in both streams, although this was much less obvious when differences in flow rate were taken into account (spores/sec. compared with spores/l).

The month by month concentrations of the more abundant aquatic hyphomycetes recorded from filtered stream water are given in Table 5.9 and the average species composition of each stream is given in Table 5.10. Some differences between the two streams were

Fig. 5.24: Spore concentration in stream water from Hakarimata and Reid's Ck, as measured by filtering, followed by the stream temperatures measured at the same time (current temperature, and maximum and minimum attained in the month since the previous sample). Spore concentration is given as spores/l and as spores passing a given point per second (x 10^{-2}), i.e. spores/l x stream discharge. (Note that the spore concentration for January (1 month) = 9118 spores/l, off the scale of the graph.) The time scale (months) used corresponds to that of the leaf bag experiment, so that 0 = December 1982, 1 = January 1983 etc.

Fig 5.24

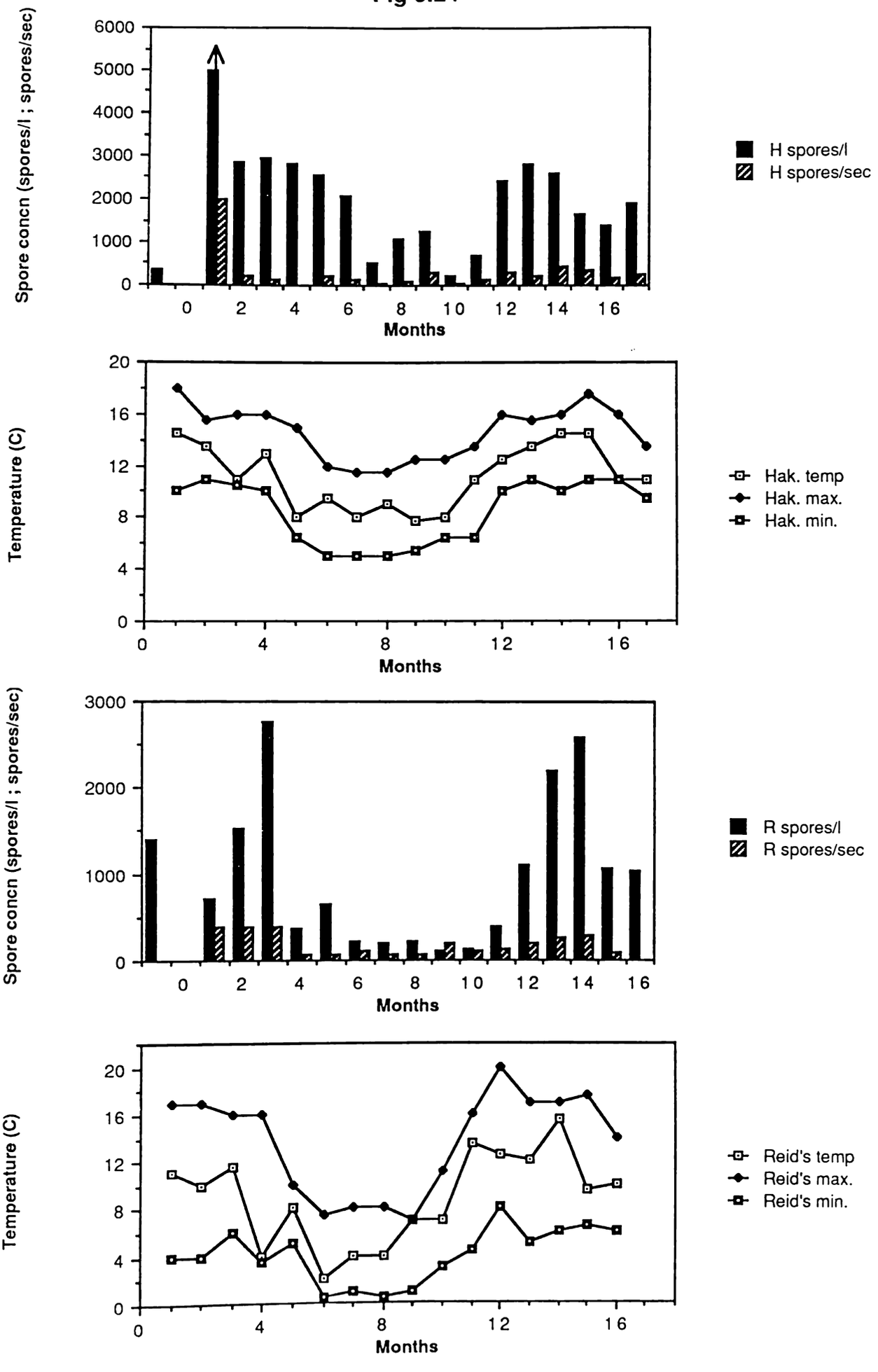
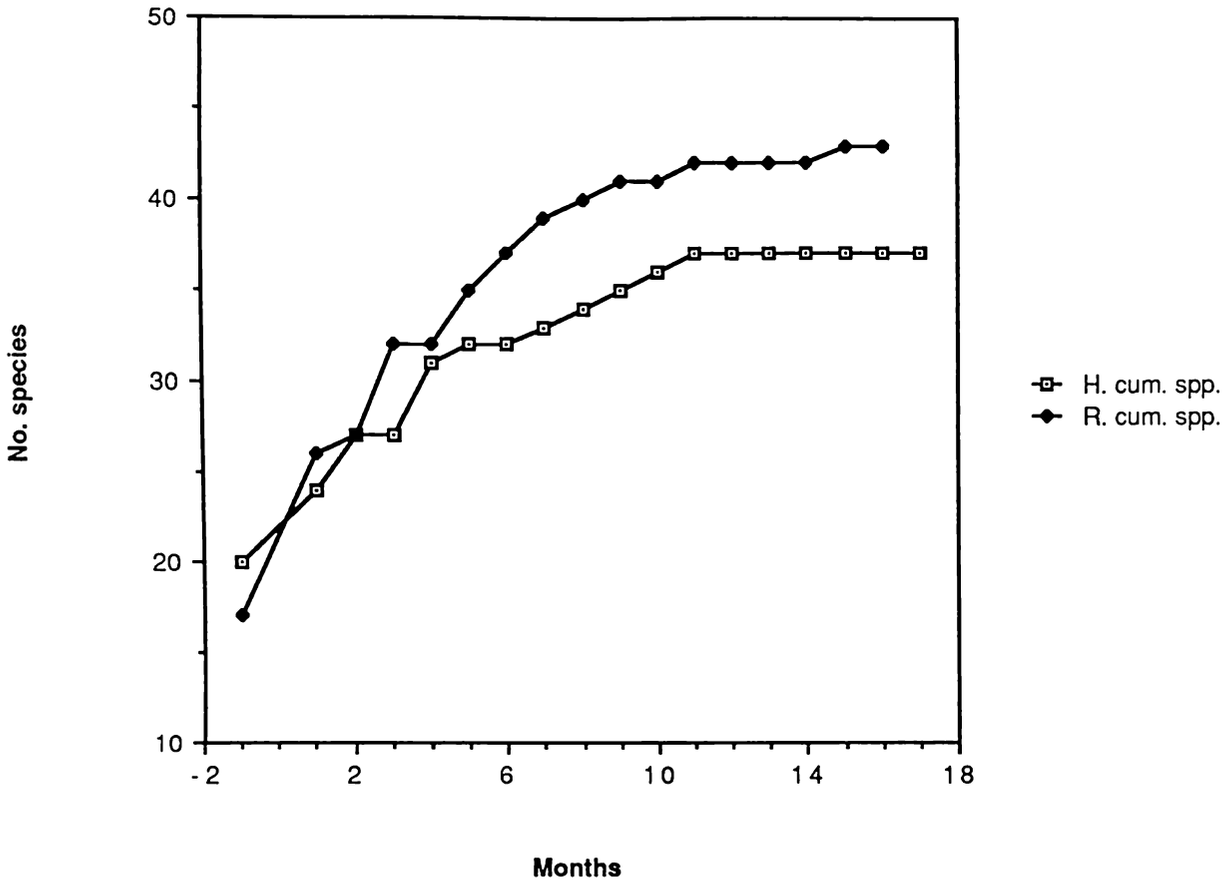

Spore concn (spores/l ; spores/sec)
6000
5000
4000
3000
2000
1000
0
0 2 4 6 8 10 12 14 16
Months
H spores/l
H spores/sec
Temperature (C)
20
16
12
8
4
0
0 4 8 12 16
Months
Hak. temp
Hak. max.
Hak. min.
Spore concn (spores/l ; spores/sec)
3000
2000
1000
0
0 2 4 6 8 10 12 14 16
Months
R spores/l
R spores/sec
Temperature (C)
20
16
12
8
4
0
0 4 8 12 16
Months
Reid's temp
Reid's max.
Reid's min.

Fig. 5.25: Cumulative number of species recorded from the Hakarimata and Reid's Ck stream filters. Time scale used corresponds to that of the leaf bag experiment (0 = December 1982, 1 = January 1983 etc).

Fig 5.25



immediately apparent. The dominant species in Hakarimata were *L. ovalis* sp. ined. and *Lunulospora* spp., together comprising 68 % of the spora on average. Only *Lunulospora* spp. comprised greater than 10 % of the total spora in Reid's Ck (41 %), but there were a larger number of species of around 5 % of the total compared to Hakarimata. More aquatic hyphomycete species were recorded from Reid's Ck than Hakarimata, as shown in Fig. 5.25 (cumulative number of species against number of samples).

Some seasonal differences between species were also apparent. Most of the more abundant species (Table 5.9) showed higher spore concentrations in summer than winter, while a number of species did not show any seasonal difference in spore concentration. Thus for comparisons between species relative abundance (%) was more useful. As relative abundance only *Lunulospora* spp. (both streams), Unidentified sp. 1 (Hakarimata) and probably *A. filiformis* (Reid's Ck) were more abundant in summer, while *T. gracilis* (Reid's Ck), s/r type 1(2) (Reid's Ck), and possibly *A. acuminata* (Hakarimata) and *A. tetracladia* (Reid's Ck) were more abundant in winter. *C. aquatica* appeared to be more abundant in spring in Reid's Ck. Thus although fewer seasonal differences were apparent from the stream filters those that were seen agreed well with the results from the leaf bag leaves (5.1.6c).

Table 5.9: Stream spora measured monthly by filtering stream water from the Hakarimata stream and Reid's Ck. The number of spores/l for each major species (species comprising the five most abundant species in any sample) is given first, followed by the percentage of total spore concentration for that month that this represents.

		Month																
	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M
HAKARIMATA																			
A. acuminata No.	18		0	100	67	22	22	13	44	78	75	5	28	66	17	66	53	0	11
%	(5)		(0)	(4)	(2)	(1)	(1)	(1)	(9)	(7)	(6)	(2)	(4)	(3)	(1)	(2)	(3)	(0)	(1)
Alatospora sp.	26		200	100	33	29	33	94	19	0	50	8	11	22	134	111	40	45	22
	(7)		(2)	(4)	(1)	(1)	(1)	(4)	(4)	(0)	(4)	(4)	(2)	(1)	(5)	(4)	(2)	(2)	(1)
A. crassa+longiss.	0		0	4	4	45	22	67	6	11	40	19	28	0	67	44	93	11	22
	(0)		(0)	(<0)	(<0)	(2)	(1)	(3)	(1)	(1)	(3)	(9)	(4)	(0)	(2)	(2)	(6)	(1)	(1)
C. aquatica	0		267	33	100	157	22	94	3	11	26	9	6	22	33	89	13	67	56
	(0)		(3)	(1)	(3)	(6)	(1)	(5)	(1)	(1)	(2)	(4)	(1)	(1)	(1)	(3)	(1)	(5)	(3)
C. longibrachiata	37		802	33	67	13	33	27	11	44	36	1	11	44	25	111	13	0	67
	(10)		(9)	(1)	(2)	(1)	(1)	(1)	(2)	(4)	(3)	(1)	(2)	(2)	(9)	(4)	(1)	(0)	(4)
F. penicillioides	0		267	33	100	157	22	94	3	11	26	9	6	22	33	89	13	67	56
	(0)		(3)	(1)	(3)	(6)	(1)	(4)	(1)	(1)	(2)	(4)	(1)	(1)	(1)	(3)	(1)	(5)	(3)
L. ovalis	52		1870	1460	1470	1418	868	908	217	343	408	67	189	332	568	243	173	278	189
	(14)		(21)	(51)	(50)	(50)	(34)	(44)	(42)	(32)	(32)	(31)	(14)	(14)	(20)	(9)	(10)	(20)	(10)
Lunulospora spp.	26		3808	830	935	678	267	601	33	122	203	48	256	1506	1319	1394	638	768	1303
	(7)		(42)	(29)	(32)	(24)	(10)	(29)	(6)	(11)	(16)	(22)	(37)	(62)	(47)	(53)	(38)	(55)	(68)
Mycocentrospora sp	26						11		3	11	8					22		11	11
	(7)						(<1)		(1)	(1)	(1)					(1)		(1)	(1)
T. marchalianum	7		0	20	20	0	0	13	3	33	0	5	6	89	84	44	13	22	0
	(2)		(0)	(1)	(1)	(0)	(0)	(1)	(1)	(3)	(0)	(2)	(1)	(4)	(3)	(2)	(1)	(2)	(0)
T. chaetocladium	22		401	100	33	29	11	27	22	33	71	11	11	22	17	66	53	22	22
	(6)		(4)	(4)	(1)	(1)	(<1)	(1)	(4)	(3)	(6)	(5)	(2)	(1)	(1)	(2)	(3)	(2)	(1)
Unident. sp. 1	4		534	0	0	35	33	13	0	0	22	0	33	133	268	177	53	78	22
	(1)		(6)	(0)	(0)	(1)	(1)	(1)	(0)	(0)	(2)	(0)	(5)	(6)	(10)	(7)	(3)	(6)	(1)
Unident. s/r 1(2)	18		134	66	33	0	0	80	0	33	47	4	0	22	0	22	67	0	56
	(5)		(2)	(2)	(1)	(0)	(0)	(4)	(0)	(3)	(4)	(2)	(0)	(1)	(0)	(1)	(4)	(0)	(3)
REID'S CK																			
A. acuminata	133		67	53	178	49	58	11	15	16	2	8	17	10	44	89	78	45	
	(10)		(9)	(4)	(6)	(13)	(9)	(5)	(8)	(8)	(2)	(6)	(4)	(1)	(2)	(3)	(7)	(4)	
Alatospora sp.	0		6	0	30	0	0	6	3	2	2	0	10	19	30	44	45	33	

Table 5.9 cont.

	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M
		(0)	(1)	(0)	(1)	(0)	(0)	(3)	(2)	(1)	(2)	(0)	(3)	(2)	(1)	(2)	(4)	(3)	
A. crassa+longiss.		0	17	66	14	18	8	6	10	28	0	13	7	10	0	22	0	0	
		(0)	(2)	(4)	(1)	(5)	(1)	(3)	(5)	(14)	(0)	(10)	(2)	(1)	(0)	(1)	(0)	(0)	
A. filiformis		55	61	133	156	18	21	8	1	7	3	13	14	86	221	200	33	33	
		(4)	(8)	(9)	(6)	(5)	(3)	(4)	(1)	(3)	(2)	(10)	(4)	(8)	(10)	(8)	(3)	(4)	
A. tetracladia		44	28	40	0	13	4	0	12	7	4	8	2	0	44	33	11	0	
		(3)	(4)	(3)	(0)	(4)	(1)	(0)	(6)	(3)	(3)	(6)	(1)	(0)	(2)	(1)	(1)	(0)	
C. aquatica		100	22	80	148	9	21	8	10	19	15	11	45	86	89	67	22	0	
		(7)	(3)	(5)	(5)	(2)	(3)	(4)	(5)	(9)	(13)	(8)	(12)	(8)	(4)	(3)	(2)	(0)	
C. longibrachiata		33	6	53	30	9	0	0	7	10	0	0	2	48	74	144	67	45	
		(2)	(1)	(4)	(1)	(2)	(0)	(0)	(4)	(5)	(0)	(0)	(1)	(4)	(3)	(6)	(6)	(4)	
Filosporella sp.		0	0	0	29	0	0	17	4	11	0	0	14	0	0	22	0	0	
		(0)	(0)	(0)	(1)	(0)	(0)	(8)	(2)	(5)	(0)	(0)	(4)	(0)	(0)	(1)	(0)	(0)	
F. penicillioides		11	17	0	0	4	0	6	0	0	0	3	0	86	15	0	22	22	
		(1)	(2)	(0)	(0)	(1)	(0)	(3)	(0)	(0)	(0)	(2)	(0)	(8)	(1)	(0)	(2)	(2)	
Lunulospora spp.		387	84	691	631	133	67	8	6	8	13	29	86	477	1285	1614	623	557	
		(28)	(12)	(45)	(23)	(36)	(10)	(4)	(3)	(4)	(11)	(21)	(22)	(43)	(58)	(62)	(58)	(54)	
T. gracilis		0	11	0	0	4	13	17	22	23	5	8	14	10	44	44	33	11	
		(0)	(2)	(0)	(0)	(1)	(2)	(8)	(12)	(11)	(4)	(6)	(4)	(1)	(2)	(2)	(3)	(1)	
T. elegans		22	17	53	0	4	21	11	2	6	8	0	24	19	59	22	11	0	
		(2)	(2)	(4)	(0)	(1)	(3)	(5)	(1)	(3)	(7)	(0)	(6)	(2)	(3)	(1)	(1)	(0)	
T. chaetocladium		11	6	0	30	9	0	0	4	5	2	3	5	0	89	44	11	56	
		(1)	(1)	(0)	(1)	(2)	(0)	(0)	(2)	(2)	(2)	(2)	(1)	(0)	(4)	(2)	(1)	(5)	
T. myrti		0	33	66	96	0	4	0	1	0	0	0	0	10	15	11	0	0	
		(0)	(5)	(4)	(3)	(0)	(1)	(0)	(1)	(0)	(0)	(0)	(0)	(1)	(1)	(<1)	(0)	(0)	
Triposporina sp.		0	15	0	17	0	0	0	3	0	2	0	0	0	0	00	0	0	
		(0)	(2)	(0)	(6)	(0)	(0)	(0)	(2)	(0)	(2)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	
Unident. s/r 1(2)	)		0	53	45	13	39	19	18	14	12	11	26	0	15	22	11	45	
	)	144	(0)	(4)	(2)	(4)	(6)	(9)	(10)	(7)	(10)	(8)	(7)	(0)	(1)	(1)	(1)	(4)	
Unident. s/r 1(3)	)	(10)	50	146	171	44	78	39	0	14	5	3	29	57	30	11	45	132	
	)		(7)	(10)	(6)	(12)	(12)	(18)	(0)	(7)	(4)	(2)	(7)	(5)	(1)	(4)	(4)	(13)	
Unident. s/r 2		22	167	0	483	0	0	0	22	7	0	0	0	19	44	33	0	0	
		(2)	(23)	(0)	(18)	(0)	(0)	(0)	(12)	(3)	(0)	(0)	(0)	(2)	(2)	(1)	(0)	(0)	

Table 5.10 Average stream spora from Hakarimata and Reid's Ck, and from the stream survey streams grouped according to riparian vegetation type (northern podocarp-hardwood, southern podocarp-hardwood, mixed podocarp-hardwood + beech, beech, and alpine). The number of samples or streams these were obtained from are given first, followed by the mean and standard deviation for total spore concentration (spores/l) in each group, then the percentage of this derived from each fungal species recorded.

	Streams						
	Hak.	Reid	N p-h	S p-h	p-h+b	beech	alpine
No. samples	18	17	6	6	6	6	4
Spores/l	2190 /1965	979 /862	2334 /1194	1810 /1106	2572 /3431	861 /910	34 /33
A. acuminata	1.80	5.28	3.51	3.37	1.79	14.79	14.63
Alatospora sp.	2.56	1.44	2.23	2.49	6.08	3.79	4.88
A. crassa+longiss.	1.33	1.47	0.26	0.98	0.53	1.66	0
A. filiformis	0.14	6.52	1.76	8.40	0.57	0.83	0
A. tetracladia	<0.05	1.55	0.26	2.13	0.61	4.50	8.94
C. sphagnorum	0	0	0	0	0	0.12	0
C. filicladia	0.09	0	0	0	0	0	0
Ceratosporium sp.	0	0	0	4.25	0.30	0	0
C. aquatica	2.61	4.55	0.90	1.56	0.49	0.47	0
C. longibrachiata	4.13	3.21	2.53	1.56	2.81	3.08	3.25
Culic. aquatica	0	0.41	0	0	0	0	0
D. aquatica	0	0.10	0.13	0	0.08	<0.12	0
E. microaquatica	0.09	0	0.26	0	0	0	0
Filosporella sp.	0.19	0.62	0.26	0.47	0.42	1.42	0
F. penicillioides	2.66	1.14	8.83	2.80	1.71	3.20	19.51

Table 5.10 cont.

	Hak.	Reid	S p-h	N p-h	p-h+b	Beech	Alpine
F. eccentrica	0	0.10	0	0	0.04	0	8.13
Gyoerffyella sp.	0	0.21	0	0.05	0	0	0
H. stellatacula	0	0.31	0.47	0	0	0.36	0
H. campanulata	0	<0.10	0	0	0	0	0
L. uniinflata		<0.01	0.26	0	<0.04	0.12	0
H. lugdunensis	0	0.21	0	0.10	0	0.36	0
L. aquatica	0.05	0.72	0.21	2.13	0.72	2.60	0
L. ovalis	29.11	0	18.29	1.92	0.68	0	0
Lunulospora spp.	38.83	40.80	37.40	27.85	40.61	24.85	0
Mycocentrospora sp	0.28	<0.10	0.26	0	0.11	0.36	0
Pleuropedium sp.	0	<0.10	0	0	<0.04	<0.12	0
T. gracilis	0	1.55	0	0	0	0.47	0.81
T. elegans	<0.05	1.66	0	5.29	5.25	5.21	0
T. marchalianum	0.90	0.10	1.29	1.56	0.53	<0.12	0
T. setigerum	0.09	<0.10	0.47	0	0	0	0
Tetracladium sp.	0.14	<0.10	0	0.16	0	0.71	0
Tetracladium sp 2	0.19	0.10	0.34	0.14	0	0	0
T. chaetocladium	2.56	1.35	0.34	1.61	2.05	0.17	0
T. splendens	0	<0.10	0	0	0	0	0
Tricladium sp.	0	0.10	0	0	0	0	0
T. acuminatus	0.05	0.31	1.03	0.67	0.08	0	0
T. myrti	0	1.45	<0.04	0.36	0.11	0	0
Triposporina sp	0	1.14	0	0	0	0	0
V. aurantiaca	0.05	0	0	0	0	0	0
Unident. sp. 1	3.70	0	6.17	0.93	0	0	0
Unident. sp. 2	0	1.14	0	0	0	1.07	0
Unident. sp. 3	1.71	0	0	0	0	0	0
Unident. s/r 1(2)	1.52	2.48	3.60	17.43	27.76	8.88	0

Table 5.10 cont.

	Hak.	Reid	N p-h	S p-h	p-h+b	Beech	Alpine
Unident. s/r 1(3)	0.71	6.21	0.90	1.50	0.76	9.82	7.32
Unident. s/r 2	1.23	4.87	0	0.83	0.61	1.89	0
Unident. s/r 4	0.28	<0.1	0.64	0	0.27	0	0
Unident. s/r 10	0.76	1.04	0.77	1.87	0.76	2.96	0
Unident. s/r 12	0.19	0	0	0	0	0	2.44
Unident. s/r 14	0.05	0.21	0	0.36	0	2.01	9.76
Unident. s/r 15	0	0.93	0	1.04	0.84	0	3.25
Unident. s/r 18	0	0	0	0	0.57	0.24	0
Unident. s/r 19	0	0	0	0	<0.04	1.30	0
Unident. s/r 21	0.19	0	0	0	0	0	0
Unident. s/r 22	0.14	0.41	0	0.31	0.08	0.59	0.81
unident. tetrarad.	0.19	0.41	0.43	0.21	0.08	0.36	5.74
unident. sigmoid	1.33	4.14	6.17	5.39	2.66	1.18	9.76
unident. spores	0.09	1.86	0	0	0	0	0

5.1.8 Random Leaf Collections: Aquatic Hyphomycete Spore Production.

In addition to the leaf types introduced to the streams in bags, naturally occurring mixed leaf litter was collected at monthly intervals from the Hakarimata stream and Reid's Ck over a period of one year, and aquatic hyphomycete spore production measured. The purpose of these measurements, as with the stream filters, was to test for differences in species composition between the two streams and between seasons, to support the observations made from the leaf bag leaves, and to measure the sample to sample variation which could be expected in the stream survey.

The average species composition, as determined by spore production, is given in Table 5.12. The month by month results for total spore production and for species averaging 5 % or more of the total are given in Table 5.11.

Some differences in species richness and species composition between the two streams were apparent. In Hakarimata the dominant species were *L. ovalis* sp. ined., *Lunulospora* spp., and *T. chaetocladium*. Together these comprised 92.3 % of the total spores produced. In Reid's Ck *A. acuminata*, *A. filiformis*, and *Lunulospora* spp. were the dominant species, closely followed by *T. elegans* and Unidentified s/r type 1(3). Together these 5 species comprised 81.4 % of the total spore production. A total of 20 species was seen in Hakarimata compared with more than 32 in Reid's Ck. Thus there was a difference in species diversity between the two streams, as was seen from the stream filters.

Levels of spore production were generally higher in Hakarimata than Reid's Ck. Because of the difference in species composition, however, there was no marked difference in the average hyphal

Table 5.11: Spore production from naturally occurring leaves collected from Hakarimata and Reid's Ck over one year, given as mean and standard deviation of total spores/g (x 10^3), and the percentage of this contributed by each of the major species (the species comprising the five most abundant species in at least one month). The total mycelial content (m mycelium/g) where it could be calculated, is given last for each month.

	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
HAKARIMATA												
Total spores/g	3698.9 /673.6	1379.5 /1376.1	1771.8 /366.1	457.2 /465.1	1473.9 /386.3	1418.6 /359.0	1806.5 /994.6	1191.8 /273.3	559.5 /600.0	162.0 /51.6	349.1 /60.1	458.3 /77.0
Alatospora sp.	0.46	0.60	0.80		0.14	0.71		1.33	1.66	0.19	0.37	
C. aquatica		0.82	0.10	0.68		0.36	0.29	1.32	1.93	0.56	0.55	0.02
E. microaquatica		0.08	0.35	3.72	0.61	1.58	2.16			2.16	0.20	
F. penicillioides	0.08	0.61	1.50	1.92	0.28							
L. aquatica	0.32										0.20	0.07
L. ovalis	19.41	13.82	15.94	49.45	31.77	46.18	17.75	24.37	71.74	89.57	32.37	0.63
Lunulospora spp.	76.20	71.62	72.34	24.17	63.76	34.79	56.30	37.87	2.57	6.17	21.77	31.57
T. chaetocladium	2.83	12.13	7.90	19.36	0.85	15.04	23.49	21.87	2.43	0.74	1.43	1.09
Unident. sp. 1	0.31		0.89	0.46	1.28	0.86		13.06	18.37		42.65	64.67
Unident. s/r 12	0.09	0.16	0.12		0.73				0.09	0.31	0.29	
Total mycelium/g	7700	4668	5164	1765	2355	5744	7906	?	?	268	?	?
REID'S CK												
Total spores/g	87.4 /9.8	308.7 /72.0	543.4 /133.0	98.3 /55.0	33.8 /7.2	76.2 /16.2	226.1 /100.6	52.9 /6.3	240.9 /56.4	216.3 /23.6	45.8 /6.5	
A. acuminata	15.45	9.14	1.51	2.54	5.03	8.14	34.72	11.91	14.40	14.89	6.77	
A. filiformis	25.17	32.72	15.38	37.13	14.20	6.82	14.64	13.99	11.75	14.01	9.61	
A. tetracladia	3.43	0.87	1.12	1.02		0.52	2.74	3.40	4.44	1.11	6.33	
C. longibrachiata	1.26	0.29	1.45	3.26	7.69		0.09	5.48	0.42	1.02	0.87	
Lunulospora spp.	18.76	37.29	63.47	34.69	18.64	6.82	20.70	8.51	15.11	31.95	12.66	
T. gracilis		1.33	1.66	3.26	2.07	0.26	1.24	3.40	1.20	1.02	1.53	
T. elegans	10.07	7.94	5.32	5.70	7.69	8.66	5.22	10.02	13.62	3.56	16.59	
Unident. sp. 2	0	0.13	0.02	1.12	0	0	0.09	0	22.13	0.18	0	
Unident. s/r 1(2)	)	)		1.93	5.33	2.76	2.08	3.39	0.21	2.45	6.11	
Unident. s/r 1(3)	13.39	5.12	1.31	2.14	5.33	37.27	13.36	20.23	5.94	15.49	13.54	
Unident. s/r 15			1.03		21.60			1.51				
Total mycelium/g	?	3190	3430	934	?	?	3875	?	?	?	?	

Table 5.12: Spore production from randomly collected leaves from Hakarimata and Reid's Ck, and from the stream survey streams grouped according to riparian vegetation type (northern podocarp-hardwood, southern podocarp-hardwood, beech, and alpine). The number of samples or streams measured are given first, followed by the mean and standard deviation for total sporulation (x 10^3 spores/g), and the mycelial content (m mycelium/g) calculated from this. The percent of the total spore production derived from each species is then given.

	Streams					
	Hak.	Reid's	N p-h	S p-h	beech	alpine
No. samples	12	11	6	6	4	3
Spores/g (x 10^3)	1227.3 /972.3	171.3 /154.0	1027.3 /507.8	437.4 /378.2	61.6 /52.1	31.5 /26.7
Mycelium (m/g)	3759	3039	2557	2178	1008	1096
A. acuminata	0.05	11.21	0.18	0.39	7.72	34.40
Alatospora sp.	0.53	0.44	0.94	0.98	1.50	1.97
A. crassa+longiss.	0.01	0.01			0.09	
A. filiformis	0.07	18.56	0.82	3.40	2.53	
A. tetracladia		1.94		0.25	0.96	34.88
C. filicladia			0.01			
C. aquatica	0.37	0.50	0.10	0.03	0.19	
C. longibrachiata	0.01	1.17	0.26	0.32	0.22	6.61
Culic. aquatica		0.03			<0.01	
E. microaquatica	0.67		0.10			
Filosporella sp.		0.04			0.04	
F. penicillioides	0.35		3.60	3.41		1.21
F. eccentrica		1.00		0.79	0.45	3.11

Table 5.12 cont.

	Hak.	Reid	N p-h	S p-h	beech	alpine
G. entomobryoides			0.01			
Gyoerffyella sp.		0.01				
H. lugdunensis		0.14				
L. aquatica	0.09	0.59	1.39	4.00	1.69	1.05
L. ovalis	25.89	0.03	8.96			
Lunulospora spp.	56.66	35.68	81.27	64.99	11.96	0.19
T. gracilis		1.27			0.47	
T. elegans		7.41	0.02	16.49	31.84	1.46
T. marchalianum	0.09		0.35	0.31		
T. setigerum	0.01	0.01	0.06	0.23		
Tetracladium sp.	<0.01			0.40		
T. chaetocladium	9.75	1.49	0.83	1.05	0.37	0.25
T. splendens		0.01				
T. acuminatus		0.10	0.11		0.13	0.19
T. myrti	<0.01	0.14		0.01	0.19	
Unident. sp. 1	5.19		0.86	1.73		
Unident. sp. 2		2.87		0.28	0.19	
Unident. s/r 1(2)	0.07	1.34	0.11	2.19	31.3	
Unident. s/r 1 (3)		8.55	0.02	0.05	6.69	4.61
Unident. s/r 2		0.03			0.37	
Unident. s/r 4	<0.04	0.07		0.05	0.06	
Unident. s/r 10		0.01		0.20	0.04	
Unident. s/r 12	0.14	0.48	0.02	0.14	0.06	
Unident. s/r 14		0.16		<0.01	0.37	
Unident. s/r 15		0.08				
Unident. s/r 18		0.14			0.21	
unident. tetrarad.		1.31			0.09	
unident. sigmoid		0.12				0.10
unident. spores		0.81				
Dermat sigmoids		1.58				

lengths that these spore counts represent (Table 5.12).

Some species were more abundant (spores/g) in Reid's Ck, while others occurred in Hakarimata at higher levels as measured by % of total spore production (Table 5.12). Species which were more important in Hakarimata were *L. ovalis* sp. ined., *F. penicillioides*, *T. marchalianum*, *T. chaetocladium*, Unidentified sp. 1 and 2, and to a lesser extent *A. pulchella* and *Lunulospora* spp. Species which were more important in Reid's Ck (and at least 1 % of the total) were *A. acuminata*, *A. filiformis*, *A. tetracladia*, *C. longibrachiata*, *F. eccentrica*, *T. gracilis*, *T. elegans*, Unidentifed s/r type 1(2) and 1(3). Unidentifed sp. 2 appears to have been more abundant in Reid's Ck, but because it is not easily separated from *L. ovalis* sp. ined. as detached spores it is possible that it was missed in the abundance of spores of *L. ovalis* sp. ined. in Hakarimata. These results agree well with the patterns of distribution seen in the leaf bag leaves (5.1.6d), although the distribution of a few species was more clear from either randomly collected or leaf bag leaves.

There was no seasonal pattern apparent in either stream (Table 5.11) because although the highest total spore production in both streams was recorded from leaves collected in summer or early autumn (respectively, December in Hakarimata, March in Reid's Ck), the intervening values varied considerably. Because of this variability % rather than actual spore production values were preferred for examining seasonal patterns among individual species, even though the use of % is limited by the species affecting each other's apparent seasonal occurrences. There were no seasonal patterns apparent among the most abundant species (Table 5.11).

The spores of the two *Lunulospora* spp. recorded together (*L. cymbiformis* and *L. curvula*) overlap in size and shape and were not counted separately for most of the field experiments. However an estimation of their relative importance in Hakarimata was obtained from the random leaf collections. Their importance on the leaves in the leaf bags appeared to be the same when *Lunulospora* was prominent, that is before late decay. Based on the presence or absence of a distinct bulge on the spore the following percentages of the *Lunulospora* spp. spores definitely belonged to *L. cymbiformis*:

18/4/83	96 %
15/8/83	95 %
20/12/83	96 %
13/4/84	59 %
19/2/86	19 %

The remaining spores either belonged to *L. curvula* or were indistinguishable from it.

From this it is clear that for most of the leaf bag experiment *L. cymbiformis* was the dominant species, but that towards the end of the experiment identification of the spores became more uncertain. By February 1986 *L. curvula* was clearly the dominant species. At this time two neighbouring streams (Firewood Ck and the Waipa River) were also dominated by *L. curvula*. No explanation could be found for the change in species composition in Hakarimata.

In Reid's Ck during the leaf bag experiment the majority of the *Lunulospora* spores were not clearly identifiable as one or the other species, though only a small proportion (4 -21 %) definitely belonged to *L. cymbiformis*.

On two occasions particular leaf types were collected as naturally occurring leaves in order to examine their potential as substrates for aquatic hyphomycetes. Total spore production figures for mosses from Hakarimata stream were low: 1.3×10^3 spores/g compared with the range of figures for randomly collected leaf litter from Hakarimata (Table 5.11). This might be in part due to the moss sample consisting of a mixture of living and dead material. A total of 10 identifiable species was seen.

The total spore numbers recorded from *Egeria densa* (collected from the Waipa River) were relatively low (205.6×10^3 spores/g), although within the range seen on randomly collected leaves in Hakarimata. Once again, this may be in part due to the presence of living, and therefore uncolonised, material in the sample. Seven species were seen.

Thus although aquatic hyphomycetes were found on both mosses and *Egeria* neither appeared to be good substrates for these fungi.

5.2: STREAM SURVEY.

A number of differences between Hakarimata and Reid's Ck were apparent from the results from the leaf bag experiment, stream filters and random litter collections given in this chapter so far. The aim of the stream survey was to test whether these differences in species composition occurred in other, similar, streams and hence represented ecologically significant differences in the distribution of aquatic hyphomycete species. The range of undisturbed New

Zealand stream types examined for qualitative and quantitative differences in the aquatic hyphomycete flora and its potential importance in leaf decay was also extended to include alpine and South Island streams. Davis and Winterbourn (1977) reported that very little colonisation of mountain beech leaves occurred in a South Island stream. The leaf bag experiment and associated random leaf collections in Reid's Ck have found that this could not have been a substrate effect, but the possibility of a stream effect remained, i.e. that aquatic hyphomycete abundance might vary between streams.

The sampling was carried out over 3 years during the period of December to March. Twenty eight streams were sampled in native vegetation types, from southern Westland to Auckland. Mixed hardwood or podocarp -hardwood riparian vegetation occurred at 12 of these, podocarp -hardwood plus beech at 6 of them, beech forest at 6 of them, and alpine vegetation (i.e. vegetation above the treeline) at 4. The factors recorded for each stream (Appendix 15) were: location, date of sampling, latitude, altitude, discharge or estimated size of stream, riparian vegetation type. In addition pH, temperature, and BOD5 (Biological Oxygen Demand over 5 days, a measure of trophic status) were recorded for most of the North Island streams. Spore counts from filtered stream water were made for all streams, and spore production was measured from incubated leaf samples for most streams. Full data is given in Appendix 15. The average species composition, average total spore concentrations from stream water, and average mycelial content obtained from leaf collections are given in Tables 5.10 and 5.12, following grouping of the streams according to vegetation type.

Total spore concentrations in the stream water varied from 6 to 9328 spores/l, a slightly wider range compared with 200 -7348 in Hakarimata and 58 -2438 in Reid's Ck, measured in the course of the

leaf bag experiment. Similarly, where leaf collections were made spore productivity ranged from 3.3 x 10^3 to 1613.5 x 10^3 spores/g/day, compared with 157.6 -1767.5 x 10^3 recorded from naturally occurring leaf litter from Hakarimata and 36.5 -312.0 x 10^3 from Reid's Ck. The streams with lowest spore concentrations and spore productivities were all alpine streams, suggesting the abundance of aquatic hyphomycetes might correlate with riparian vegetation.

Dividing the streams on the basis of vegetation type gave the following ranges of spore concentration and productivity:

	Spore Concentration (spores/l)	Spore Productivity (spores/g/day x 10^3)
Alpine:	6 -70	3.3 -56.5
Beech:	192 -2591	23.2 -137.9
Podocarp -hardwood plus beech:	241 -9328	
P. -hardwood:	896 -4001	63.5 -1613.5

Thus there was some overlap between streams of differing riparian vegetation types, except for alpine and beech, and alpine and podocarp -hardwood streams, with respect to spore concentrations. Average values are given in Tables 5.10 and 5.12. Differences in spore productivity could result from either less colonisation by aquatic hyphomycetes, perhaps as a result of leaves being retained in-stream for a shorter or longer period, or by a difference in species composition, such that the dominant species had different mycelium/spore values. Differences in spore concentration in stream water were most likely to have resulted from either differences in the amount of litter in the stream, or in the spore productivity of that litter.

The difference in spore productivity of aquatic hyphomycetes

between Hakarimata and Reid's Ck, as measured in the leaf bag experiment, continued in other streams with similar vegetation types (podocarp -hardwood and beech), and there appeared to be a rough correspondence between riparian vegetation type and aquatic hyphomycete abundance. As with Hakarimata and Reid's Ck, however, the difference in mycelial content from the litter samples was much less great: beech streams averaged 1008 m/g, compared to 2368 m/g for podocarp -hardwood streams, whereas the average spore productivity of litter from beech streams was less than a tenth of that recorded from podocarp -hardwood streams. Similarly, the difference between the mycelial content of alpine litter samples and those from other stream types was small compared to the difference in spore production, although much of the calculated mycelial content of alpine litter in particular derived from species for which only an estimated mycelium/spore values was available. However, on the basis of these values it seems that differences in species composition were responsible for much of the difference in spore productivity between streams with differing riparian vegetation. These results suggest that very few streams sampled in the stream survey would differ from Hakarimata or Reid's Ck in terms of the potential importance of aquatic hyphomycetes in leaf decay. The exceptions are alpine streams and fast-flowing South Island beech streams, which tended to show slightly lower spore productivities. These streams may correspond to the stream studied by Davis and Winterbourn (1977) when they concluded that aquatic hyphomycetes did not play a large part in the decomposition of beech leaves in New Zealand streams.

Some part of the differences in spore concentrations between streams with different riparian vegetation types may result from the spore productivity of the litter in them, rather than the amount of

litter present, but this work cannot distinguish how much. In effect, however, both will decrease in response to reductions in stream stability, for example, and spore concentration can be considered a measure of the occurrence of conditioned litter in a stream.

The number of species seen in a stream ranged from 3 to 22 on the stream filters, and 6 to 20 from leaf samples. The smallest numbers of species were recorded from alpine streams. This may have related to the small number of spores seen in total in the samples, as higher numbers of species were seen on the leaf samples with higher spore productivity from the same (alpine) habitat. Thus in alpine streams the number of species ranged from 3 -9 on the stream filters and 6 -12 on the leaf samples. In beech streams the range was 15 -22 (average 17.8) on filters and 10 -20 on leaves (average 14.3). In beech plus podocarp -hardwood streams the range was 15 -21 species on the filters. In podocarp -hardwood and hardwood streams the range was 11 -21 (average 16.1) on filters and 9 -17 on leaves (average 13.1). Thus the pattern of slightly more species found in Reid's Ck compared to Hakarimata (Fig. 5.25) was apparently continued in other streams with the same type of riparian vegetation.

The dominance of a single species varied from 20 % to 69 % of the total spore numbers on the stream filters, and from 24 % to 93 % of the spore production from the leaf collections. This also varied between streams of different riparian vegetation types, averaging 39 % for filters of podocarp -hardwood streams, 33 % for mixed beech plus podocarp streams, and 25 % for beech streams. Spore production from leaf collections averaged 61 % dominance by a single species in podocarp -hardwood streams compared with 25 % in beech streams. This followed the same pattern found in Hakarimata compared with

Reid's Ck in the leaf bag experiment.

Both the common and the occasional species found in all the streams were from the same range of species as were seen in Hakarimata and Reid's Ck in the leaf bag experiment, with the exception of a few very rare species. The absence of a particular species from an individual stream probably resulted from the improbability of a single sample containing the whole flora (Fig. 5.25).

Thus treating the results as presence/absence data would yield very few, if any, differences between stream types, with any genuine inter-stream differences probably occurring as differences in abundance only.

The variation in the levels of dominant species seen in the stream filters (Table 5.9) and randomly collected leaves (Table 5.11) of the leaf bag experiment indicates the high variability occurring between samples from the same site. Thus techniques of analysis which measured the similarity between individual streams based on quantitative values in order to group them, that is ordination and classification techniques, failed to work with the stream survey data. Similar streams, representing the same habitat, did not necessarily have the same dominant species. Moreover, whichever of the group of potentially dominant species was in fact dominant, usually dominated by a large %.

The best method of assessing differences between stream types was to group them according to a variable and compare the frequency with which the species in them were either dominant or among the 5 most abundant species, using the frequencies obtained from Hakarimata and Reid's Ck as the standards for assessment. In this way the most significant variables with regard to aquatic hyphomycete distribution could be determined. The average

occurrence of more minor species in the grouped streams could then be compared to that in Hakarimata or Reid's Ck.

In the leaf bag experiment *Lunulospora* spp. was the dominant species in 44 % of the stream filters in Hakarimata, and 71 % of them in Reid's Ck (together 58 %). It was in the top 5 most abundant species in 100 % of the filters from Hakarimata, and in 82 % of them from Reid's Ck (on average 91 % of the two streams together). If *Lunulospora* spp. was present at the same levels as Hakarimata and Reid's Ck in all stream types then it would be expected to be dominant in about 16 of the 28 samples (i.e. streams) and be in the top 5 species in about 25 of them. The actual figures were 18 and 20. This suggests that very few of the streams differed significantly from Hakarimata or Reid's Ck with regard to the importance of *Lunulospora* spp. in the flora. One obvious group was that of the 4 alpine streams, in which *Lunulospora* spp. was never dominant or in the top 5 species. Excluding these, *Lunulospora* spp. was dominant in 75 % of the streams and in the top 5 species in 83 % of them. These figures are very close to those for Reid's Ck.

The % of samples in which *Lunulospora* spp. were dominant in Hakarimata was lower than that for Reid's Ck, due to the frequency of dominance of *L. ovalis* sp. ined., which was apparently absent from Reid's Ck. *L. ovalis* sp. ined. was the dominant species in 56 % of the stream filters and in the top 5 species in 100 % of the filters in Hakarimata. In the stream survey *L. ovalis* sp. ined. was dominant in only one stream and in the top 5 species in only 9 streams, indicating a real difference in occurrence between streams. If only streams with podocarp -hardwood riparian vegetation are considered the frequency of occurrence in the top 5 species rose to 67 %, which was still quite low compared with Hakarimata. Dividing

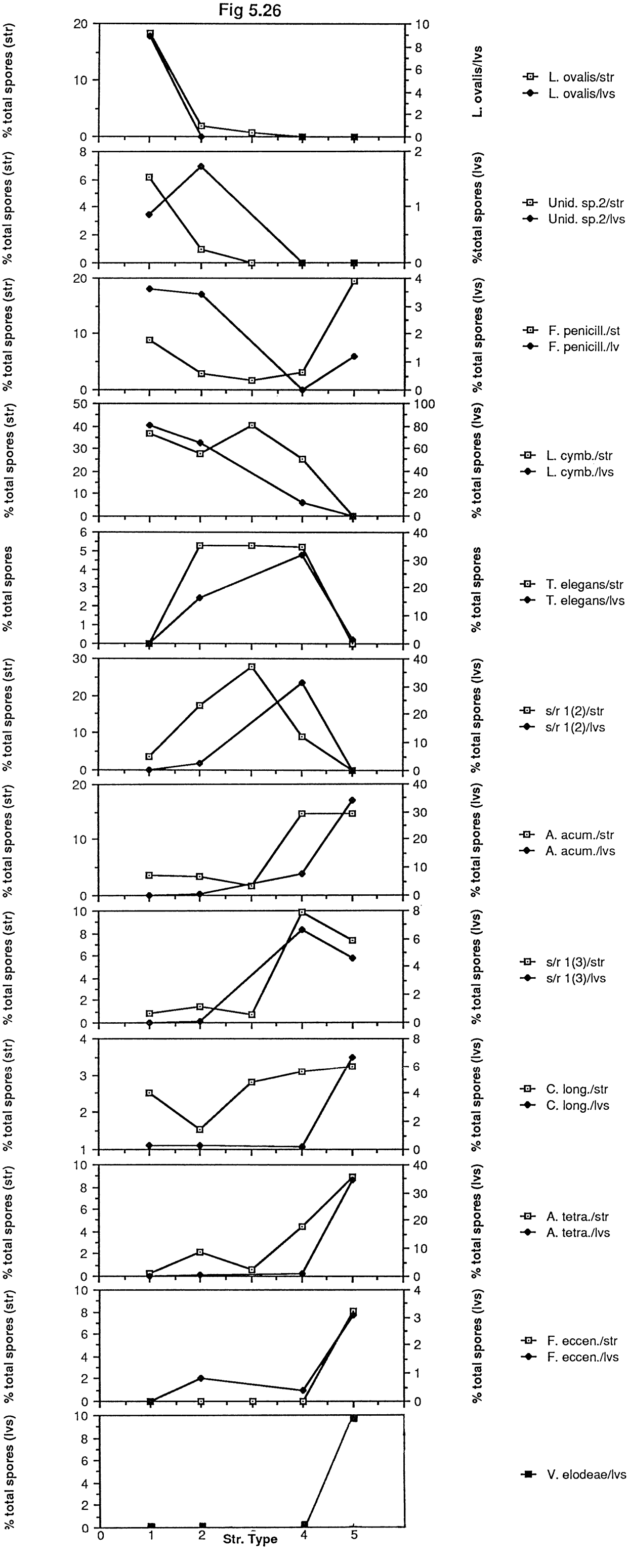
the podocarp -hardwood streams into two groups on the basis of latitude, with the cutoff point at around 38° S, between the Rangitukia stream (Pirongia) and the Waihora (Pureora), resulted in a northern podocarp -hardwood group, more like Hakarimata, in which *L. ovalis* sp. ined. occurred in the top 5 species in 83 % of the streams (although still dominant in only 17 %). In the southern group, as in the podocarp -hardwood plus beech and the beech streams (including Reid's Ck), *L. ovalis* sp. ined. never occurred in the top 5 species.

The average spore concentrations and % compositions in the stream water for all species in these grouped streams are shown in Table 5.10. The sporulation figures from the leaf collections similarly grouped are given in Table 5.12, as average spores/g and % species composition. Many of the more important species varied in abundance between the streams, so that the streams can be seen as an ecological continuum of types when grouped according to riparian vegetation and latitude into northern podocarp -hardwood, southern podocarp -hardwood, podocarp -hardwood plus beech, beech, and alpine streams. This is shown in Fig. 5.26. Relatively few species showed a uniform importance in all the stream types, namely, *Alatospora* spp., *L. aquatica*, *T. chaetocladium*, and perhaps *C. longibrachiata*. Other species showed distribution patterns ranging from abundance in northern podocarp -hardwood streams only to abundance in alpine streams only. Both spore production from leaf collections and spore concentration on the stream filters showed the same pattern in most cases, in spite of differences in relative abundance.

BOD5 and pH did not vary greatly among the streams sampled (Appendix 15) and, probably for this reason, no correspondence was found between BOD5

Fig. 5.26: Changes in species composition between stream types with different riparian vegetation types. Stream types are: 1 = Northern podocarp-hardwood, 2 = Southern podocarp-hardwood, 3 = mixed podocarp-hardwood and beech, 4 = beech, 5 = alpine vegetation. The aquatic hyphomycete species shown are: *L. ovalis* sp. ined., Unidentified sp. 2, *F. penicillioides*, *Lunulospora* spp., *T. elegans*, Unidentified sigmoid/rod type 1(2), *A. acuminata*, Unidentified sigmoid/rod type 1(3), *C. longibrachiata*, *A. tetracladia*, *F. eccentrica*, and *V. elodeae*. The average % of total spore concentrations in filtered stream water from each stream type, and the average % of total spore production from leaf samples (where these were collected) are given for each species. (Note that *V. elodeae* did not occur in measurable numbers on any of the stream filters, but was seen in some leaf samples.)

Fig 5.26


% total spores (str)
% total spores (lvs)
L. ovalis/str
L. ovalis/lvs
Unid. sp.2/str
Unid. sp.2/lvs
F. penicill./st
F. penicill./lv
L. cymb./str
L. cymb./lvs
T. elegans/str
T. elegans/lvs
s/r 1(2)/str
s/r 1(2)/lvs
A. acum./str
A. acum./lvs
s/r 1(3)/str
s/r 1(3)/lvs
C. long./str
C. long./lvs
A. tetra./str
A. tetra./lvs
F. eccen./str
F. eccen./lvs
V. elodeae/lvs
Str. Type

or pH and the aquatic hyphomycete flora. Thus the most significant factor determining aquatic hyphomycete distribution was apparently riparian vegetation type, in combination with latitude. However, a direct effect of the vegetation itself is unlikely to have occurred, as the leaf bag experiment showed there was no difference in the flora of kamahi and other species occurring in podocarp -hardwood forest compared with beech. Riparian vegetation corresponded roughly to altitude and thus to temperature and possibly other factors.

5.3: TESTING INDIVIDUAL FACTORS AFFECTING DISTRIBUTION.

In the leaf bag and stream survey work above, a pattern of species distribution among streams (relating to altitude and latitude) was found and this interacted with the seasonal occurrence of the same species. Temperature was an obvious connecting factor between latitude, altitude, and season, and could thus be important in determining the distribution of species detailed in Section 2. The temperature relations of a number of species are examined in pure culture here.

5.3.1 Effect of temperature on vegetative growth.

Trinci (1971) showed that the radial growth rate of a fungus was directly related to the specific growth rate in submerged culture when comparing growth at different temperatures. Seventeen species of aquatic hyphomycetes were grown on malt agar at some or all of

the following temperatures: 1, 5, 13, 20, 25, and 30 C. Two or three plates of each, with two colonies on each, were used, and two radii measured on each colony at weekly intervals over one month to obtain an average growth rate. The results are given in Table 5.13.

Table 5.13: Average radial growth rates (mm/day) of 17 species on 1.5 % malt agar at various temperatures. The symbols following some of the species names (89, 65, 352, 1 & 6, and TC1) refer to isolates obtained previously (Aimer and Segedin, 1985b). Species without them were derived from either the Hakarimata stream, or Reid's Ck. Those specified (Hakarimata isolates) represent recent isolates, tested later than the others.

	Temperature					
	1 C	5 C	13 C	20 C	25 C	30 C
A. moniliformis (246)	0	0.18	0.41	0.57	0.38	-
A. pulchella	-	-	0.36	-	-	-
A. filiformis	0.003	0.15	0.26	0.48	0.61	-
A. longissima	-	0.34	0.64	1.04	1.24	0.09
A. tetracladia (89)	0.14	0.50	0.93	1.17	0.55	0
(Reid's Ck isolates)	0.25	0.52	0.78	1.08	0.15	-
C. aquatica	0.06	0.50	0.70	1.44	1.50	0
Filosporella sp.	0.14	0.25	0.46	-	-	0
F. penicillioides	0	0.09	0.38	0.96	1.21	-
L. aquatica	0.08	0.26	0.56	0.82	0.80	0
L. curvula (352)	-	0	0.65	0.98	-	-
(Hakarimata isolates)	-	-	1.20	-	-	-

Table 5.13 cont.

	Temperature					
	1 C	5 C	13 C	20 C	25 C	30 C
L. cymbiformis (1 & 6)	-	0	0.49	0.59	0.44	0
(Hakarimata isolates)	-	-	0.80	-	-	-
L. ovalis sp. ined.(65)	-	0.16	0.48	0.62	0.67	-
(Hakarimata isolates)			1.16			
T. marchalianum (Hakarimata isolate)			0.77	-	-	-
T. chaetocladium (TC1)	0.04	0.33	0.65	1.11	0.88	0
(Hakarimata isolates)	-	-	1.52	-	-	-
T. splendens	0.09	0.36	0.36	0.82	0.80	-
V. elodeae	0.22	0.66	1.00	1.47	1.45	-
V. aurantiaca (Hakarimata isolate)			0.34	-	-	-

Five species showed peak growth rate at 25 C, and seven at 20 C. Thus all species tested were mesophiles, though showing optimum growth rates at the low end of the mesophile range (20 -45 C, Sharp, 1978). Most species tested showed no growth at 30 C. Below 5 C growth rates slowed considerably in most cases, only a few species making appreciable growth at 1 C. *A. tetracladia* and *V. elodeae* were notable exceptions, showing greater growth at 1 C than ~~most~~ other species, which may explain why these species were relatively more abundant in alpine streams (Fig. 5.27).

The higher growth rates observed in the "Hakarimata isolates" compared with those obtained from other isolates may relate to either of two factors: the age of the isolate or the source of the malt agar. The "Hakarimata isolates" were more freshly isolated and were grown on malt agar that was a mixture of commercial and

laboratory-prepared (from malt extract) compared to purely commercial malt agar.

The amount of variation to be found between different isolates of the same species was illustrated by the four isolates of *A. tetracladia*, one from the Nihotupu stream (89) and the rest (averaged) from Reid's Ck. Further such comparisons would be necessary to determine whether this variation was random or some sort of local adaptation to warmer and cooler stream types.

The natural habitat of aquatic hyphomycetes has frequently been stated to be clean, flowing streams but little or no previous work exists on the response of aquatic hyphomycetes to dissolved organic pollution or low oxygen conditions. Although the stream survey was limited to clean, undisturbed streams two small experiments were undertaken to examine the potential for growth in other stream types, that is, in streams with different levels of dissolved organic pollution, and under low oxygen conditions.

5.3.2 Effect of stream nutrient levels on aquatic hyphomycete abundance.

The aim of these experiments was to determine the effect of dissolved nutrient levels on aquatic hyphomycete abundance in leaves, and thus the possibility of aquatic hyphomycetes being sensitive to organic pollution of streams. The leaves were collected from the Hakarimata stream and incubated in different nutrient concentrations for a long enough period for microbial populations to have stabilised. In the first experiment 2 -3 g dry weight of leaf litter was incubated in 1 l flasks containing 750 ml

of one of five different solutions:

1) 2 g/l yeast extract, reduced to 1 g/l after several weeks;

2) full strength mineral solution;

3) 10 % mineral solution;

4) 1 % mineral solution;

5) deionised water.

The flasks were kept still, unaerated, at 13 C, the incubation solution being changed every week, for six weeks. At this time the leaves were removed, washed, and sporulation measured using the standard method, after 2, 3, 6, 9, 12, and 15 days.

The condition of leaves in the different treatments rapidly became visibly different. The flasks of yeast extract were frequently very smelly, with a gel -like layer at the surface after a week during the first few weeks of the experiment (when using 2 g/l yeast extract). The leaves were still slimy at the end of the experiment, and the solution cloudy. The leaves from the 100 % mineral nutrient solution appeared to be more decomposed than the others by the end of the experiment (although no weight loss measurements were made). They were not slimy, but developed a milky, powdery deposit on them. Leaves in the weaker solutions were indistinguishable from each other in appearance, and all appeared very clean.

The total spore production recorded following the incubation period is given in Table 5.14 and the results for the most abundant individual species, in the same experiment, are given in Table 5.15.

Table 5.14: Total spore production from leaves following two months incubation in either 1 g/l yeast extract, 100 %, 10 %, or 1 % mineral nutrient solution, or deionised water. Total spores/g/day ($\times 10^3$) are given as mean/standard deviation.

	Incubation solution				
	Yeast	100 % N.	10 % N.	1 % N.	Deionised
Day 2	3/1	86/64	676/49	1168/399	919/289
Day 3	15/5	109/56	667/141	691/208	611/202
Day 6	35/13	118/76	491/37	345/67	160/27
Day 9	75/16	137/56	580/68	277/110	23/55
Day 12	93/22	146/78	545/35	230/69	145/12
Day 15	119/1	269/240	368/45	249/104	125/20

Differences in the normal "peak" sporulation (at Day 2 - standard method, 2.1.4) were apparent between the treatments. Aquatic hyphomycete abundance, as measured by spore production, increased significantly from the yeast extract treatment to the 1 % mineral nutrient treatment. There was no difference between 1 % mineral nutrient and deionised treatments.

Subsequent spore production also showed a distinct pattern, with numbers increasing with time in the yeast extract treatment, remaining constant in 100 % mineral nutrient treatment, and decreasing with time in 10 %, 1 % mineral nutrient, and deionised water treatments. The lowest nutrient treatments showed the greatest decrease, similar to that of spore numbers from leaves collected from Hakarimata and incubated immediately on collection (4.3.3).

Thus some factor in the high nutrient treatments seemed to have inhibited aquatic hyphomycete growth, and removal of the nutrient

solution allowed them to begin recovering. It would appear that vegetative growth and sporulation were ocurring at the same time, so that as the mycelial content increased, spore production also increased in the high nutrient treatments. In contrast, the more fixed mycelial content in the low nutrient treatments was used up through spore production, which slowly declined.

The inhibitory effect of the high nutrient treatments could have been due to inhibition by competing microorganisms which were favoured by high nutrient conditions, or possibly to the decreased oxygen concentrations resulting from increased respiration from microorganisms generally. The effect of differences in oxygen availability between treatments was examined in the second trial. However, some evidence of bacterial presence was obtained in this trial from preliminary experiments for the selective inhibition of respiration technique (6.4.2). Some of these experiments used leaves from the yeast extract and 100 % mineral nutrient treatments in fresh nutrient solutions of each to test the technique. It was found that about 90 % of the respiration was bacterial in these high nutrient treatments. Unfortunately no results were obtained for the less enriched treatments, but in leaves collected fresh from streams (and showing similar aquatic hyphomycete sporulation values to those seen in the 1 % mineral nutrient and deionised water treatments) generally slightly less than 50 % of the total respiration was attributable to bacteria. The total respiration rate (bacteria plus fungi) recorded from leaves in 100 % mineral nutrient solution was about twice that of leaves from deionised water. Thus it would appear that bacterial colonisation of the leaves was considerably stimulated by the high nutrient treatments, coinciding with inhibition of the aquatic hyphomycetes.

Table 5.15: Spore production by the dominant species on leaves following two months incubation in either 1 g/l yeast extract, 100 %, 10 %, or 1 % mineral nutrient solution, or deionised water (sporulation measured on day 2 only). Spores/g/day are given as mean/standard deviation. "L. cymb." = *L. cymbiformis*, "T. chaet." = *T. chaetocladium*, "L. ovalis" = *L. ovalis* sp. ined., "C. longibr." = *C. longibrachiata*.

Species	Incubation solution				
	Yeast	100 % N.	10 % N.	1 % N.	Deionised
L. cymb.	51/ 53	11,681/ 16,185	300,880/ 65,816	754,022/ 375,967	746,839/ 238,663
T. chaet.	1,449/ 568	21,541/ 14,465	89,759/ 9,371	115,331/ 34,646	52,502/ 20,088
L. ovalis	417/ 420	2,526/ 4,375	121,019/ 71,056	41,283/ 25,828	51,445/ 12,363
C. aquatica	1,002/ 1008	13,310/ 5,923	32,833/ 8,506	49,388/ 27,867	41,732/ 33,801
C. longibr.	49/ 47	10,733/ 7,075	105,826/ 32,381	181,640/ 42,903	26,693/ 24,749

A change in species composition with decreasing nutrient concentration is clear from Table 5.15, despite the high variation recorded in most cases. *T. chaetocladium* and *C. aquatica* were the dominant species following the yeast extract treatment, and at least

co-dominants following 100 % mineral nutrient treatment. In the other treatments *L. cymbiformis* became increasingly dominant, surpassing *T. chaetocladium* and *C. aquatica* in the 10 % mineral nutrient treated leaves (44 % of total spores), and comprising 81 % of the total spores in the deionised water-treated leaves. The differences presumably relate to the degree of tolerance of the species to the factor(s) causing inhibition of aquatic hyphomycetes in high nutrient conditions, that is, low oxygen or competitive effects.

In the second trial the solutions were aerated to remove, or at least reduce, the oxygen limitation imposed by high nutrient conditions. Only three nutrient solutions were used: 1 % and 100 % mineral nutrient solution, and 1 g/l yeast extract solution. Replicate lots of 0.3 g of leaves were placed in 150 ml of each solution, in 250 ml flasks. A pressurised air supply was fed to the flasks via pasteur pipettes, at the lowest possible flow rate to give a steady stream of bubbles but avoid moving the leaves round and causing them to fragment. In addition to replacing the incubation fluid at weekly intervals the flasks were checked regularly and any fluid loss due to evaporation topped up with deionised water. The leaves were incubated for six weeks before sporulation was determined, using the standard method, after 2, 4, 6, 8, and 12 days. The results are given in Table 5.16 as total spore production, and in Table 5.17 as spore production by the dominant species.

Table 5.16: Total spore production from leaves following six weeks incubation in either 1 g/l yeast extract, 100 %, or 1 % mineral nutrient solution (aerated). Total spores/g/day (x 10^3) are given as mean/standard deviation.

	Incubation solution		
	Yeast Extract	100 % Nutrient	1 % Nutrient
Day 2	20/10	691/209	1611/647
Day 4	144/44	1036/221	2594/1118
Day 6	60/18	366/162	553/219
Day 8	75/13	380/334	338/116
Day 12	88/48	297/154	538/180

Table 5.17: Spore production by the dominant species on leaves following six weeks incubation in either 1 g/l yeast extract, 100 %, or 1 % mineral nutrient solution (sporulation measured on day 4 only, the peak in production). Spores/g/day (x 10^3) are given as mean/standard deviation.

Species	Incubation solution		
	Yeast	100 % N.	1 % N.
L. cymbiformis	5/7	612/179	1329/486
L. ovalis sp. ined.	2/2	60/45	222/132
F. penicillioides	11/2	8/7	13/8
T. chaetocladium	<1/1	0	26/33

The results differed noticeably from those in the first trial in that there was less difference in spore production between the treatments. The peak figures for total spore numbers (Day 4) compare closely with those obtained in the first experiment (Table

5.14) for the 1 % mineral nutrient-treated leaves, but for the yeast extract- and 100 % mineral nutrient-treated leaves were about ten times greater. Thus there was no statistically significant difference between 1 and 100 % mineral nutrient-treated leaves (t test), although the yeast extract-treated leaves showed significantly lower sporulation than the other two. A large part of the inhibition of aquatic hyphomycetes in the first trial was therefore evidently due to low oxygen concentrations. The remaining difference between the yeast extract and 1 % mineral nutrient treatments may have been attributable to insufficient aeration. However, some type of competitive or inhibitory interaction between microorganisms cannot be ruled out in this case.

T. chaetocladium and *C. aquatica* were present at levels too low to distinguish whether they were less inhibited than other species, as in the first trial. However *F. penicillioides* appeared to follow a similar pattern to that of *T. chaetocladium* and *C. aquatica* in the first trial (Table 5.15), being the dominant species in the yeast extract-incubated leaves (57 %) but only 14 % of the total spores in 1 % mineral nutrient-incubated leaves. As in the first trial, *Lunulospora* spp. dominated the low nutrient treatment.

5.3.3 Effect of oxygen concentration on vegetative growth.

The importance of aeration to aquatic hyphomycetes, in the absence of competing microorganisms, was examined in this experiment. The most sensitive species to nutrient enrichment (and dominant species in most streams in New Zealand), *L. cymbiformis*, was chosen. Its tolerance of conditions of low oxygen was tested as the effect of oxygen concentration on respiration rate.

This experiment used mycelium from the liquid culture experiment (4.1.2), incubated in 10 g/l glucose in mineral nutrient solution. Twenty ml of mycelium plus nutrient solution were sealed in each of six 30 ml McCartney bottles. Three replicate bottles were reaerated regularly, to keep the oxygen concentration in the air above the solution greater than 10 or 15 %, while the oxygen content was allowed to decline in the remaining three bottles. The method of incubation and measurement of respiration followed that of the standard S.I.R. technique (2.4.3). The results are given in Table 5.18.

Table 5.18: Comparison between respiration rates of *L. cymbiformis* mycelium grown in bottles of nutrient solution with (control) and without (low oxygen) regular reaeration to replenish the oxygen used up. Mean/standard deviation respiration rates (μg O_2/hr/bottle) of each, the ratio of the low oxygen treatment to the high, and the oxygen content of the low oxygen bottles (same as control bottles at 3 and 12 hours) are given for incubation periods of 3 to 67 hours.

Time (hrs)	Control Respiration	Low O2 Respiration	Ratio	O2 concentration
3	71/28	83/17	116 %	17 %
12	55/8	53/12	96 %	14 %
20	39/13	39/40	102 %	11 %
26	80/22	21/7	27 %	8.5 %
36	75/10	26/6	35 %	7.5 %
43	81/12	19/8	24 %	6.0 %
67	68/18	5/4	7 %	4.5 %

There appears to be a fairly abrupt decrease in the efficiency of respiration at slightly under 10 % oxygen.

The method of obtaining a range of oxygen concentrations (sealing the bottles and allowing the oxygen concentration to be reduced by respiration) caused carbon dioxide levels to increase as oxygen decreased, and a small negative pressure to build up (visible at the end of the experiment when the bottles were inverted and a needle inserted through the septum in the lid: a bubble rose from it). The effect, if any, of either of these factors on respiration, independent of oxygen concentration, was not tested. Thus the present work provided a better test of the ability of *L. cymbiformis* to grow in low oxygen conditions as they occur in the field, than the measurement of a single physiological property, i.e. response to oxygen concentration. It is clear from the present work that *L. cymbiformis* is not at all tolerant of low oxygen conditions.

Thus in future work it could be expected that aquatic hyphomycetes would not be good indicators of organic pollution of streams unless it coincided with low oxygen conditions. However, other conditions, such as increased sediment, which could bury leaves and reduce the oxygen concentrations, might be expected to have an impact on aquatic hyphomycete populations.

The results given in this chapter are discussed in Chapter Eight.

CHAPTER SIX: RESULTS IV -MEASUREMENT OF THE RELATIVE IMPORTANCE OF DIFFERENT MICROBIAL GROUPS.

Introduction.

In previous work (reviewed by Bärlocher, 1985) it has been shown that aquatic hyphomycetes are active in the decay of leaves in streams, and can act as conditioning organisms, preparing leaf litter for consumption by invertebrate detritivores. In the present work aquatic hyphomycetes have been shown to occur in some abundance throughout the decay of most leaf types in a large number of New Zealand streams (Chapter 5). However, a number of other microorganisms occur in the same habitat and may perform the same functions. The likely ecological impact of the degradative capabilities of aquatic hyphomycetes therefore depends on the relative abundance of aquatic hyphomycetes compared to other microbial groups.

In order to measure the relative abundance of aquatic hyphomycetes and other microorganisms in the present work Anderson and Domsch's (1973, 1974) selective inhibition of respiration method was used, somewhat modified, to measure the relative contributions of bacteria (prokaryotes) and fungi (eukaryotes) to microbial respiration, and therefore activity, in leaves collected from streams. In the basic method respiration was stimulated by the addition of nutrients. The degree to which this stimulation was suppressed by the presence of antibacterial and antifungal antibiotics provided a measure of the relative importance of bacteria and fungi. A number of preliminary experiments were

necessary to develop the method, and to test the effectiveness of the antibiotics chosen on the growth and respiration of the target organisms. The S.I.R. (Selective Inhibition of Respiration) method was then extended in an attempt to determine the relative contributions of aquatic hyphomycetes compared with other fungi by drawing on the relationship between respiration and sporulation of aquatic hyphomycetes determined in sections 4.2.6 and 4.2.7.

A second method, involving plating of leaf pieces, was used to estimate the relative importance of aquatic hyphomycetes compared with other fungi, as measured by the frequency of detection. The method is common in studies of terrestrial fungi (e.g. Hettige, 1981), but was used here in a rather abbreviated form. No subculturing was carried out, the fungi present being detected by the presence of sporulating structures on the agar surrounding the leaf pieces following partial submersion.

6.1 SELECTIVE INHIBITION OF RESPIRATION EXPERIMENTS.

6.1.1 Technique development: Test of effectiveness of antibiotics.

Chloramphenicol was the antibacterial antibiotic, and nystatin and cycloheximide were the antifungal antibiotics chosen for the present work. The effectiveness of these against their target and against nontarget organisms was tested in three experiments.

In the first experiment, the effectiveness of the antibiotics against the entire natural microflora was tested through their influence on the frequency of isolation of bacteria and fungi from macerated leaves. The method was adapted from that used by Suberkropp and Klug (1976) to measure bacterial numbers in leaves

from a stream.

Five 0.5 cm diameter discs were cut from leaves collected from Hakarimata and ground in a Waring blender in 125 ml sterile water. Six 10 ml samples of this were used in dilution series, to test three antibiotic treatments in two agar types. The three antibiotic treatments were: control (no antibiotics), chloramphenicol (60 ppm), and cycloheximide plus nystatin (each at 50 ppm). The antibiotics were dissolved in 0.4 ml methanol, sterile water added, and the solution filter sterilised before adding to the agar. The two agars used in the test were Glucose Yeast Peptone Agar, for enumerating bacteria, and Glucose Peptone Rose Bengal Agar, for enumerating fungi.

Glucose Yeast Peptone Agar

1 g/l glucose

1 g/l peptone

0.2 g/l yeast extract

15 g/l agar

Glucose Peptone Rose Bengal Agar

10 g/l glucose

2 g/l peptone

0.5 g/l KH_2PO_4

0.5 g/l $MgSO_4.7H_2O$

0.05 g/l Rose Bengal

15 g/l agar

Three macerated leaf dilutions were tested on each agar type, using ten plates of each. The dilutions used were 10^{-4}, 10^{-5}, and 10^{-6} on GYP Agar, and 10^{-1}, 10^{-2}, and 10^{-3} on GPRB Agar. The number of

colonies on each set of plates were counted after ten days incubation at room temperature.

The counts from the most effective dilutions are given in Table 6.1.

Table 6.1: Number of colonies of bacteria and fungi per plate (mean of 10 plates/standard deviation) cultured from macerated leaves diluted to 10^{-1} in GPRB agar, and 10^{-4} and 10^{-5} in GYP agar, in the presence of antibacterial (chloramphenicol), antifungal antibiotics (cycloheximide + nystatin), or neither (control). The total number of colonies on 10 plates is given for the higher dilutions (10^{-2}, 10^{-3} GPRB, and 10^{-6} GYP agar).

Medium + Leaf dilution		Number of colonies: Bacteria	Fungi
GYP AGAR			
10^{-6}	Control	9	0
10^{-6}	Antibacterial	0	0
10^{-6}	Antifungal	1829	0
10^{-5}	Control	27.8/6.7	0
10^{-5}	Antibacterial	0.7/0.7	2
10^{-5}	Antifungal	198.3/20.7	0
10^{-4}	Control	42.4/9.8	0
10^{-4}	Antibacterial	7.5/2.8	0
10^{-4}	Antifungal	162.7/20.7	0

Medium + Leaf dilution		Number of colonies Bacteria	Fungi
GPRB AGAR			
10^{-3}	Control		12
10^{-3}	Antibacterial		1
10^{-3}	Antifungal		1
10^{-2}	Control		74
10^{-2}	Antibacterial		50
10^{-2}	Antifungal		4
10^{-1}	Control		11.7/4.0
10^{-1}	Antibacterial		13.1/4.1
10^{-1}	Antifungal		4.0/2.1

The growth of bacteria on the GYP plates was significantly inhibited (t test) by chloramphenicol. On average, 90 % inhibition occurred, and colonies which appeared on the chloramphenicol treated plates were small and slow growing compared to those on the control plates. The GYP replicates with cycloheximide and nystatin showed higher bacterial colony numbers than the controls, but closely similar numbers appeared in all three dilutions, indicating they did not derive from the leaf material, but were contaminants, possibly from the nonsterile flask in which the antifungal antibiotics were dissolved. The control numbers in the 10^{-4} dilution were somewhat fewer than were expected from the numbers in the higher dilutions. Possibly there was some inhibition between colonies at this concentration, although much higher numbers appeared in the antifungal plates.

Chloramphenicol did not inhibit or stimulate the growth of mycelial fungi. The antifungal antibiotics however gave significant

inhibition, averaging 80 % inhibition of mycelial fungi. The control and antibacterial plates in the 10^{-1} dilution showed lower colony numbers than were expected from the higher dilutions. Considered in conjunction with the similar patterns in the GYP plates (10^{-4} compared with 10^{-5} and 10^{-6}) this suggests the dilution technique may have been at fault. If this is the case it does not affect the conclusions with regard to the effectiveness of the antibiotics.

The effect of the three antibiotics on the respiration of an aquatic hyphomycete, *L. cymbiformis* was tested using mycelium, grown in liquid culture (see methods, 2.1.1). Four replicate bottles of mycelium from liquid culture were set up in the standard medium for S.I.R. experiments (2 g/l glucose and 10 % mineral nutrient solution), with 60 ppm chloramphenicol, 50 ppm cycloheximide, 50 ppm nystatin, or 50 ppm cycloheximide + 50 ppm nystatin). The control sets were identical, but lacked mycelium. No control containing mycelium but without antibiotics was set up. The standard S.I.R. method of incubation was used, and respiration measurements made at about 3 and 19 hours. The resulting respiration rates are given in Table 6.2.

Table 6.2: Effect of chloramphenicol, cycloheximide, nystatin, and cycloheximide plus nystatin on the respiration rate of *L. cymbiformis* in liquid culture over a 21 hour incubation period. Respiration is given as mean/standard deviation, mg O_2/g mycelium/hour.

	Antibiotic treatment			
	Chloramph.	Cyclohex.	Nystatin	Cyclo.+Nyst.
3 Hours	1.63/0.09	1.39/0.27	0.40/0.07	0.47/0.03
19 Hours	1.57/0.11	1.17/0.39	0.01/0.10	0.01/0.01

Nystatin gave total inhibition of existing respiration in less than 24 hours. In contrast, cycloheximide did not have a significant effect, even after 19 hours, compared to the three hour measurement of respiration, or compared to the chloramphenicol treatments. Chloramphenicol probably did not have a marked effect on respiration as there was no difference between the 3 and 19 hour measurements.

Cycloheximide and chloramphenicol act on ribosomes, inhibiting protein synthesis (Betina, 1983), so the speed at which an effect on respiration appears depends on the rate of breakdown of respiratory enzymes, or on the rate of growth. Nystatin causes impairment of the cell membrane barrier function, leading to cell death (Kerridge, 1986). Thus the difference between the two antifungal antibiotics may have been due to the length of time required to have an effect on respiration rather than a difference in susceptibility of the fungus.

In the sterile leaf experiment (4.2.8) *L. cymbiformis* was grown on sterilised kamahi, beech, and ponga leaves, and *T. chaetocladium* on kamahi and beech, for 77 days. At the end of this time four replicate subsamples of each were incubated as in the standard S.I.R. method. Thus eight sets of four replicates were set up:

uninoculated kamahi,

uninoculated beech,

uninoculated ponga leaves,

L. cymbiformis grown on kamahi,

L. cymbiformis on beech,

L. cymbiformis on ponga,

T. chaetocladium grown on kamahi,

T. chaetocladium on beech.

Three of each set were incubated with nutrients alone, while cycloheximide and nystatin (both at 50 ppm) were added to the fourth replicate. The aims of this work included measuring the rate at which fungal respiration was stimulated by the nutrient levels (glucose plus mineral nutrients) used in the standard method (developed using natural microbial populations), as well as testing the effectiveness of the antifungal antibiotics against aquatic hyphomycetes in natural substrates. The respiration rates of each are shown in Figs 6.1 and 6.1a.

The results show that aquatic hyphomycetes can and do respond rapidly to added nutrients within the time scale of the standard S.I.R. method as used with naturally occurring leaves. The antibiotic-treated inoculated leaves did not show this increase and even declined slightly. Thus aquatic hyphomycetes on natural substrates respond rapidly to antifungal antibiotics (resulting in all growth inhibited) even though they do not show the same total inhibition of respiration, in the short term, as fungi in agar discs or liquid culture. This residual uninhibited respiration may represent existing, old mycelium, although it is not clear why nystatin should apparently kill existing mycelium in agar, but not in leaves, unless the turnover rate is very different in spite of the same nutrient solution being present about each substrate.

The respiration rate of some of the antibiotic treated leaves (for example *T. chaetocladium*) increased after about 30 hours, presumably due to bacterial contamination occurring.

Fig. 6.1: Respiration rate of control leaves (sterile) and of leaves inoculated with *L. cymbiformis*, following the addition of complete nutrient solution. Three different leaf types were used (1 = kamahi, 2 = beech, 3 = ponga), the average respiration for each (from three replicates) being graphed. Antifungal antibiotics (50 ppm cycloheximide and nystatin) were also added to single bottles of each inoculated leaf type (i.e. unreplicated), the respiration of each bottle being graphed separately.

Fig 6.1

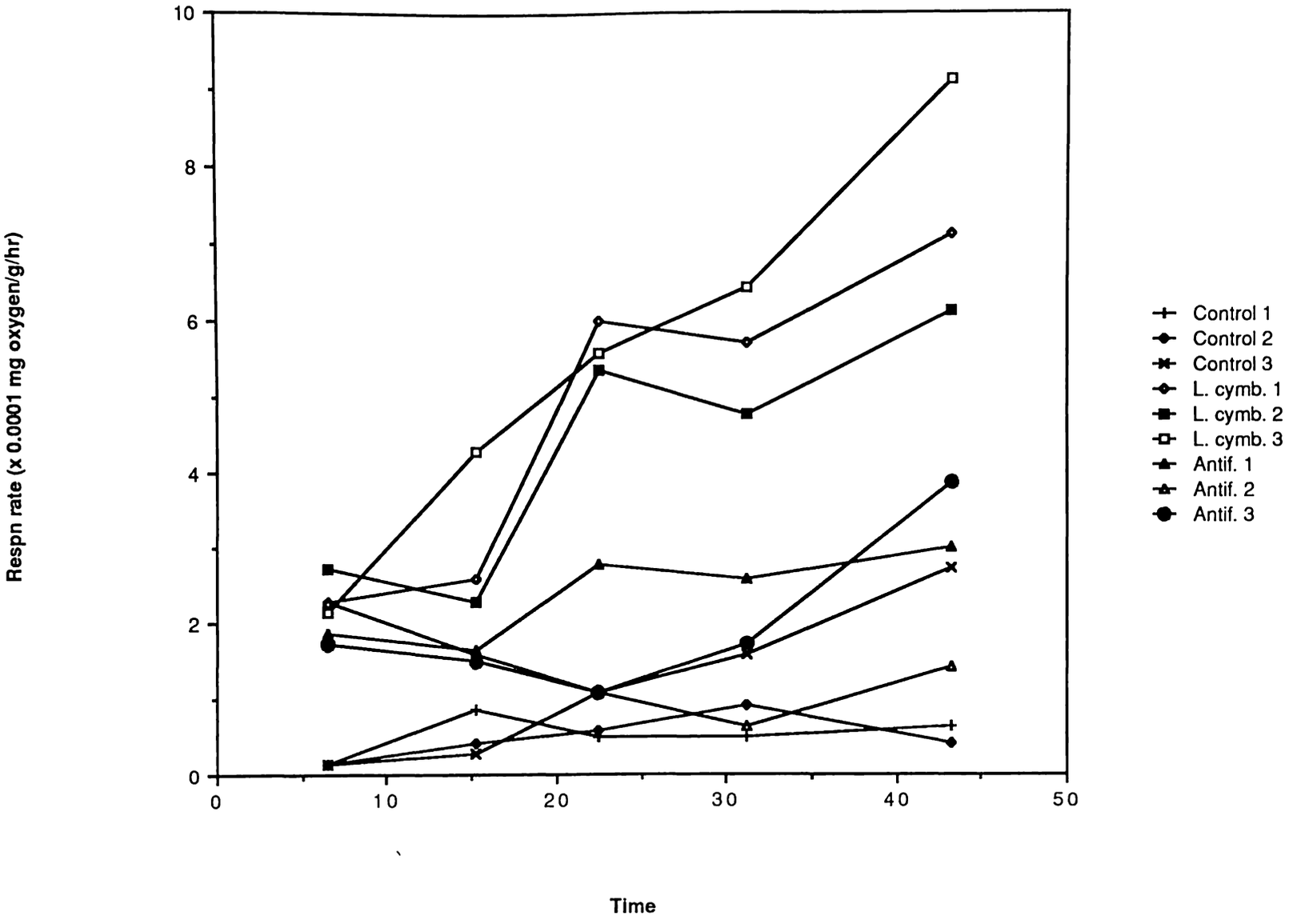
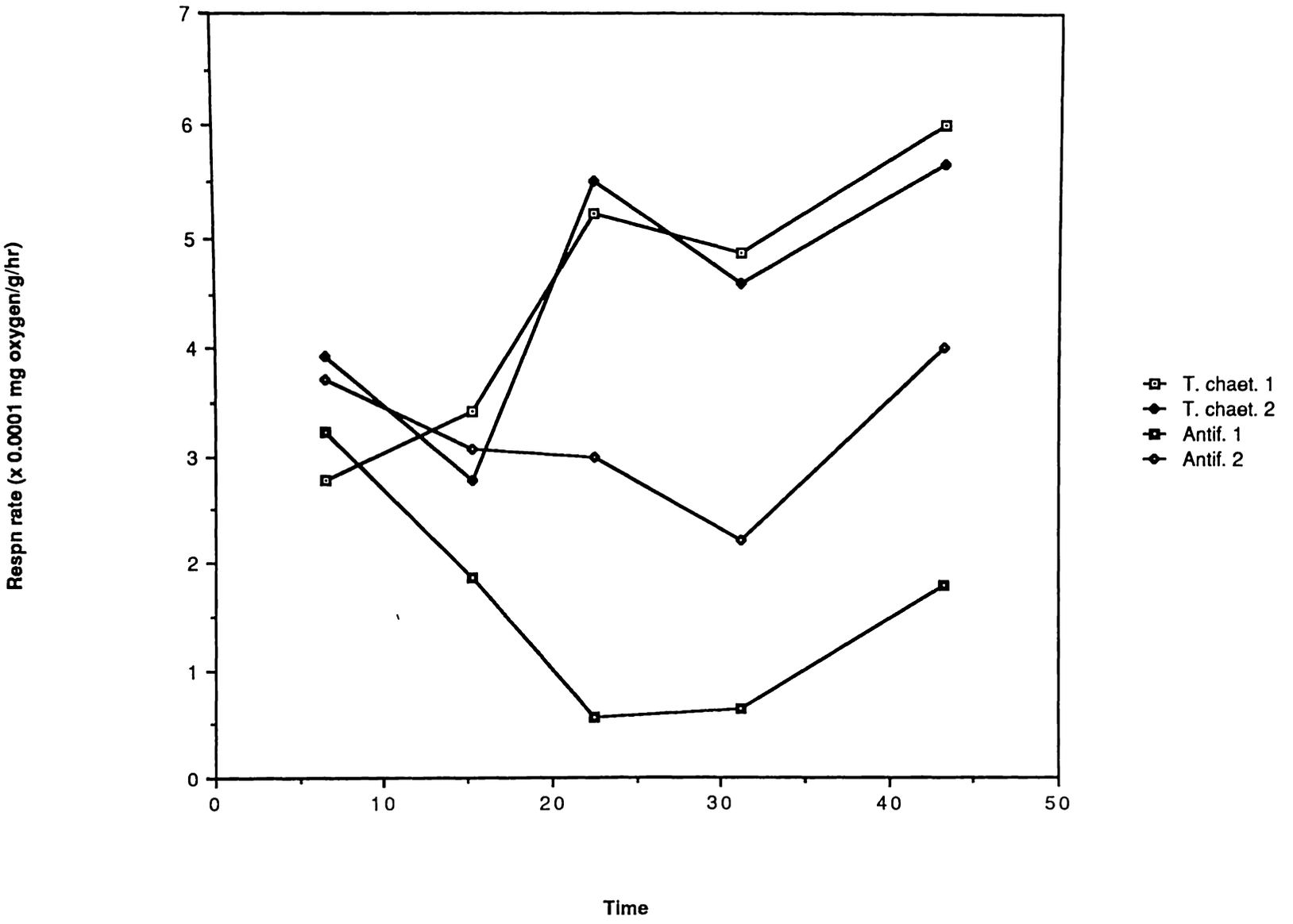

Respn rate (x 0.0001 mg oxygen/g/hr)
10
8
6
4
2
0
0
10
20
30
40
50
Time
Control 1
Control 2
Control 3
L. cymb. 1
L. cymb. 2
L. cymb. 3
Antif. 1
Antif. 2
Antif. 3

Fig. 6.1a: Respiration rate of kamahi (1) and beech (2) leaves inoculated with *T. chaetocladium*, following the addition of complete nutrient solution with (single unreplicated values) or without (average of three replicates) antifungal antibiotics. (See Fig. 6.1 for respiration rates of control, uninoculated leaves.)

6.1a


Respn rate (x 0.0001 mg oxygen/g/hr)
7
6
5
4
3
2
1
0
0
10
20
30
40
50
Time
T. chaet. 1
T. chaet. 2
Antif. 1
Antif. 2

6.1.2 Technique development: Effect of sample size, agitation, and nutrient levels.

Oxygen cells were found to be too small to use in the measurement of respiration of leaf litter: the largest sample size possible gave such extremely high variances (standard deviation 20 -60 % of the total uninhibited respiration) that even total inhibition was not statistically significant. Incubation of leaf samples in McCartney bottles, from which gas samples were withdrawn at intervals and the oxygen content analysed using a G.C., allowed larger sample sizes to be used.

The respiration rate of such samples dropped rapidly unless the bottles were shaken, or when shaken too rapidly. Keeping the bottles on a slope on the shaker appeared to give optimal rates, by increasing the surface area of the water and keeping it moving, but without moving the leaf pieces significantly. This method, together with keeping the oxygen content of the air above the water fairly high (not lower than about 15 % O_2), reduced the limiting effect of the rate of oxygen diffusion on respiration rate to a satisfactory extent.

Little or no inhibition of respiration by any antibiotics occurred with incubation of leaf samples (collected from Hakarimata stream) in stream, deionised, or other waters low in nutrients, even if incubated for up to a week. Antibiotics seemed incapable of affecting already existing respiration, but inhibited the increase in respiration when nutrients were added. Glucose and other nutrients were tested to achieve the maximum stimulation of respiration, and therefore maximum (measureable) antibiotic response. Glucose concentrations less than 0.02 % had little effect on respiration. When solutions of 0.02 %, 0.2 %, and 1 % glucose

were added, respiration of leaf pieces took two to three days to double compared with leaves in deionised water. The addition of mineral nutrients as well further stimulated respiration, and with 0.2 % glucose + 10 % mineral nutrient solution the doubling time was reduced to about one day, or at most 1.5 days. This solution was taken as the standard for incubation (2.1.6).

During these preliminary experiments it was found that storage of leaf material, even for one or two days (in stream water, in a refrigerator), caused the microflora to become unresponsive to subsequently added nutrients. The cause of this was not investigated, but in the standard S.I.R. method adopted, incubation of leaf samples (to measure respiration) was initiated on the day of collection.

6.1.3 Use of the selective inhibition of respiration technique.

The microbial respiration of five litter samples was divided into bacterial and fungal using the S.I.R. technique. Four of these samples were obtained from Hakarimata, one from each season (April, June, November, and December 1986). One collection was made from Reid's Ck (November 1986). The standard method for measuring inhibition of respiration (2.1.6) was used. That is, the leaves were chopped up and incubated in full nutrient solution (2 g/l glucose + 10 % Ruakura solution), with or without antibiotics, for two to four days, respiration (change in oxygen concentration) being measured periodically. The antibiotic treatments used were: control (no antibiotics), antibacterial (chloramphenicol), antifungal (cycloheximide and nystatin), and antibacterial + antifungal (chloramphenicol, cycloheximide, and nystatin). In the

April samples two concentrations of antibiotics were used: 50 and 200 ppm. In the June samples 50 and 250 ppm were used. No consistent increase in inhibition appeared at the higher concentrations over the time range used for the calculations, so 50 ppm antibiotics were used in subsequent experiments.

The changes in respiration rate with time in the different treatments are shown in Figs 6.2 -6.6 for each leaf collection. There was considerable variation between sequential measurements, but the normal pattern was that after a short lag period the respiration rates of the different treatments began to diverge, the control respiration increasing most rapidly, and the antibacterial + antifungal treated respiration rates remaining constant or declining slightly.

The antifungal treated bottles initially respired at a rate below that of the controls, as expected, but after between 12 and 24 hours entered a phase of rapidly increasing respiration, generally overtaking that of the controls after 24 hours. This suggests that the bacteria bloomed when the fungi were inhibited, and that in a stream, as in the control flasks, bacterial numbers could be actively suppressed by the fungi.

The bottles from the April sample were not emptied for about a week after the experiment finished. It was noted at this time that mycelial growth from the antibacterial treated leaves was very conspicuous. Growth was markedly less abundant, although still visible, in the control bottles, while the antifungal and antibacterial + antifungal bottles showed no visible growth. This suggests that the bacteria were also capable of limiting the growth of the fungi, although obviously not to the same extent as the Fig

Fig. 6.2: Respiration rate of randomly collected leaves from Hakarimata (28/4/86), following the addition of either complete nutrient solution alone (control), or complete nutrient solution with 50 ppm chloramphenicol (antib. 50), 200 ppm chloramphenicol (antib. 200), 50 ppm cycloheximide plus 50 ppm nystatin (antif. 50), 200 ppm cycloheximide plus 200 ppm nystatin (antif. 200), 50 ppm chloramphenicol plus 50 ppm cycloheximide plus 50 ppm nystatin (antib.+f. 50), or 200 ppm chloramphenicol plus 200 ppm cycloheximide plus 200 ppm nystatin (antib.+f. 200). Each treatment was replicated three times and the average graphed here. The nutrient/antibiotic solutions were replaced after 48 hrs.

Fig 6.2

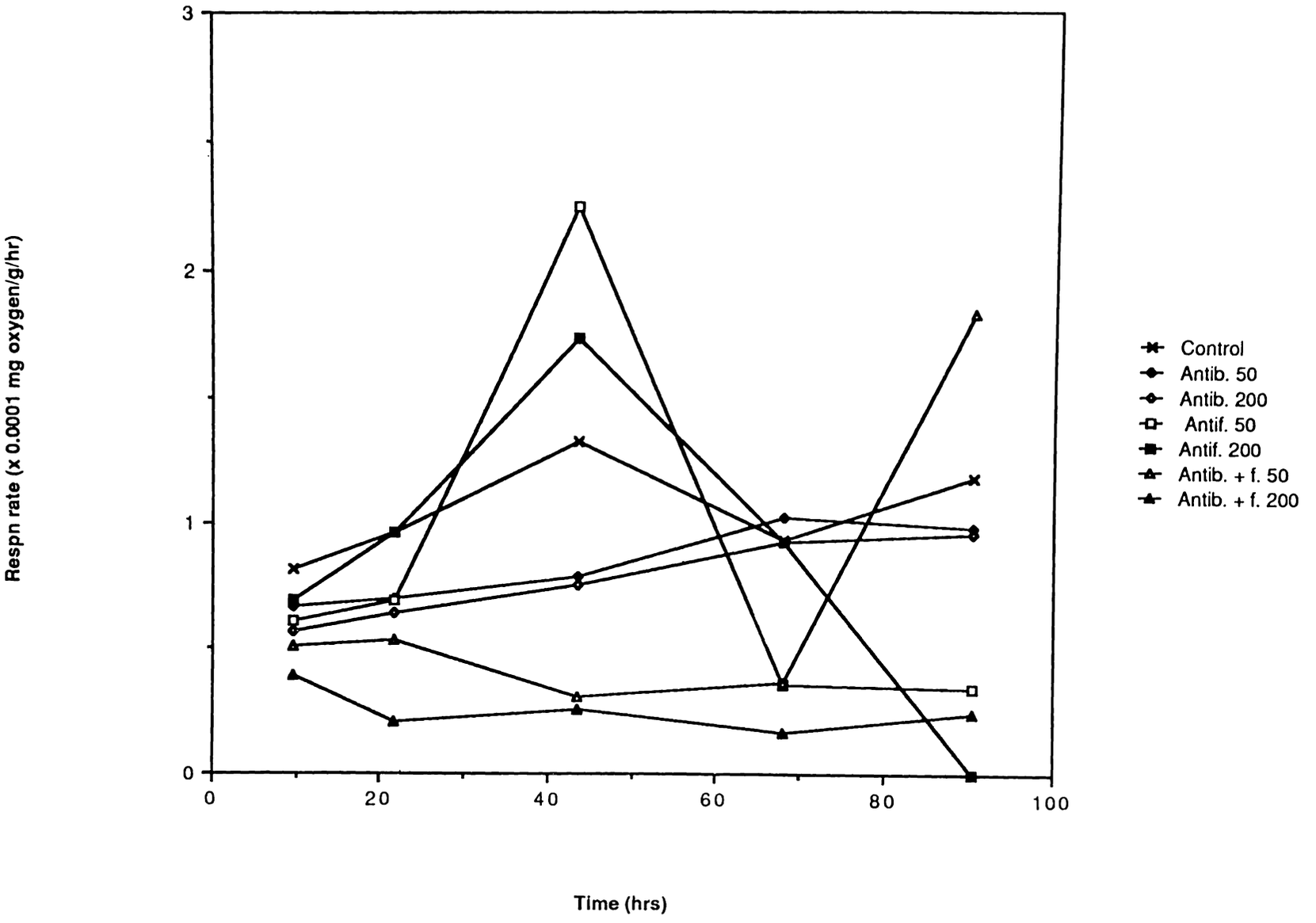

Respn rate (x 0.0001 mg oxygen/g/hr)
3
2
1
0
0
20
40
60
80
100
Time (hrs)
Control
Antib. 50
Antib. 200
Antif. 50
Antif. 200
Antib. + f. 50
Antib. + f. 200

Fig. 6.3: Respiration rate of randomly collected leaves from Hakarimata (16/6/86), following the addition of either complete nutrient solution alone (control), or complete nutrient solution with 50 ppm chloramphenicol (antib. 50), 250 ppm chloramphenicol (antib. 250), 50 ppm cycloheximide plus 50 ppm nystatin (antif. 50), 250 ppm cycloheximide plus 250 ppm nystatin (antif. 250), 50 ppm chloramphenicol plus 50 ppm cycloheximide plus 50 ppm nystatin (antib.+f. 50), or 250 ppm chloramphenicol plus 250 ppm cycloheximide plus 250 ppm nystatin (antib.+f. 250). Each treatment was replicated three times and the average graphed here. The nutrient/antibiotic solutions were replaced after 25 and 48 hrs.

Fig 6.3

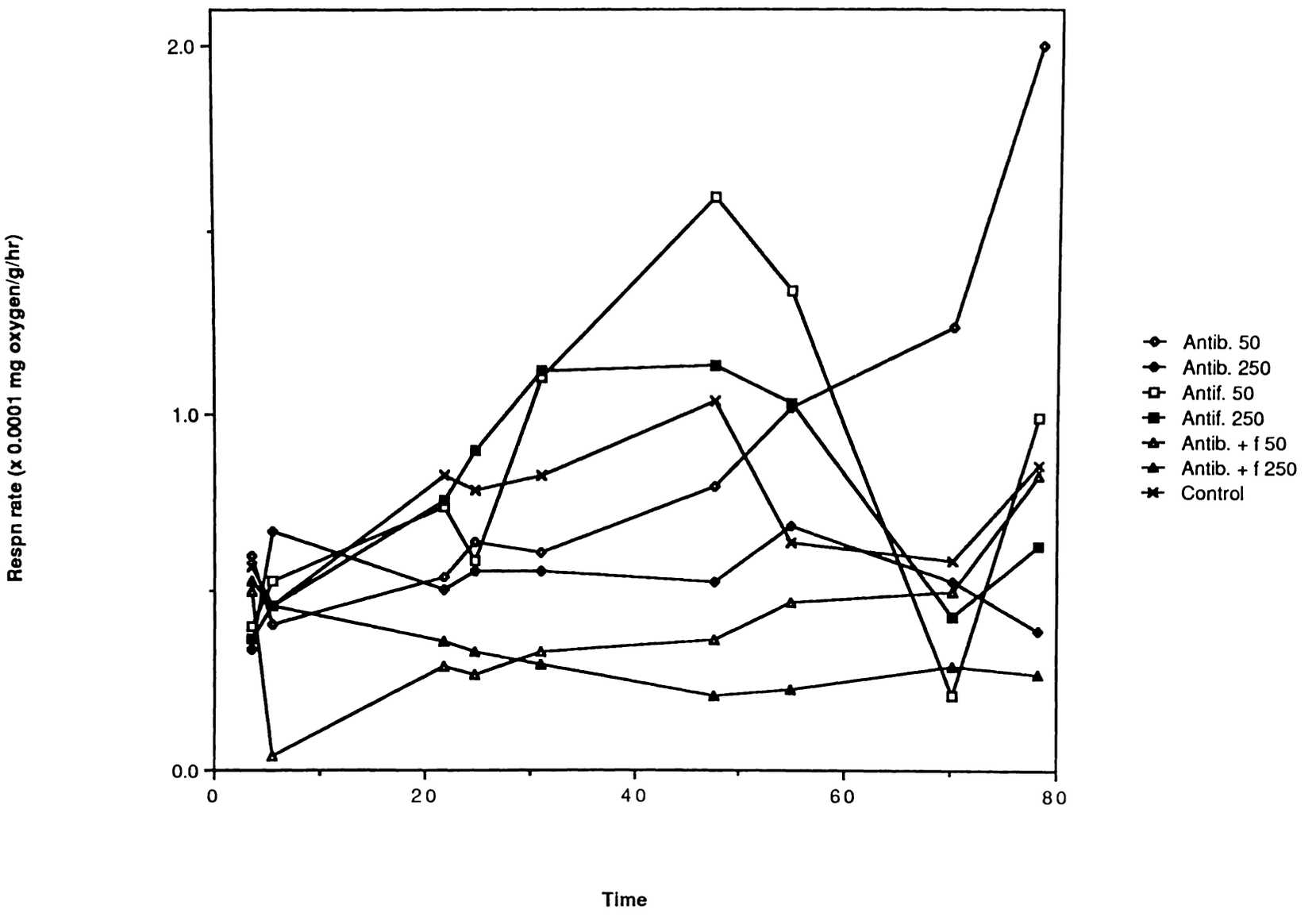

Respn rate (x 0.0001 mg oxygen/g/hr)
2.0
1.0
0.0
0
20
40
60
80
Time
Antib. 50
Antib. 250
Antif. 50
Antif. 250
Antib. + f 50
Antib. + f 250
Control

Fig. 6.4: Respiration rate of randomly collected leaves from Hakarimata (19/11/86), following the addition of either complete nutrient solution alone (control), or complete nutrient solution with 50 ppm chloramphenicol (antib. 50), 50 ppm cycloheximide plus 50 ppm nystatin (antif. 50), or 50 ppm chloramphenicol plus 50 ppm cycloheximide plus 50 ppm nystatin (antib.+f. 50). Each treatment was replicated three times and the average graphed here. The nutrient/antibiotic solutions were not replaced during this experiment.

Fig 6.4

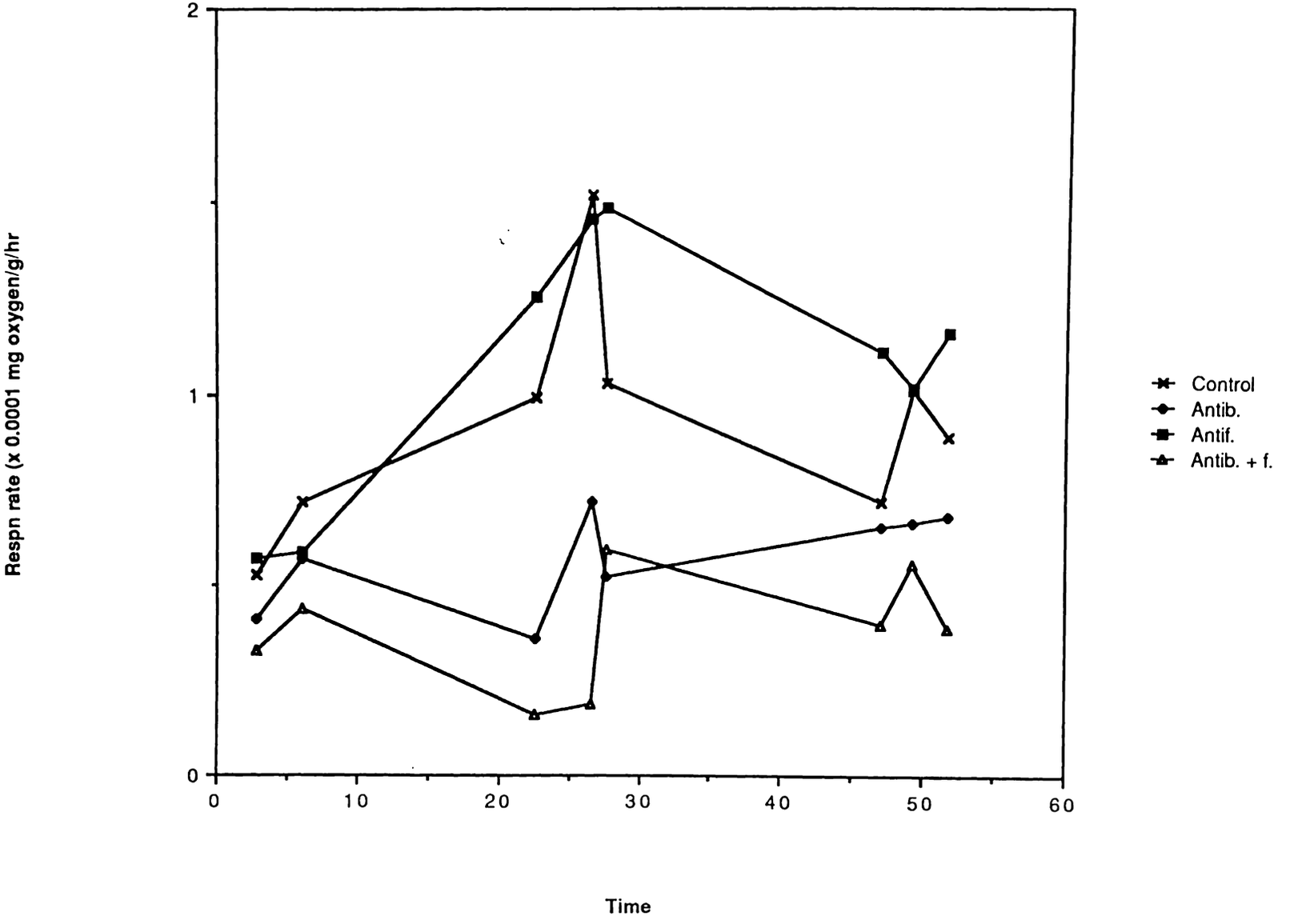

Respn rate (x 0.0001 mg oxygen/g/hr
2
1
0
0
10
20
30
40
50
60
Time
Control
Antib.
Antif.
Antib. + f.

Fig. 6.5: Respiration rate of randomly collected leaves from Hakarimata (2/12/86), following the addition of either complete nutrient solution alone (control), or complete nutrient solution with 50 ppm chloramphenicol (antib. 50), 50 ppm cycloheximide plus 50 ppm nystatin (antif. 50), or 50 ppm chloramphenicol plus 50 ppm cycloheximide plus 50 ppm nystatin (antib.+f. 50). Each treatment was replicated three times and the average graphed here. The nutrient/antibiotic solutions were replaced after 28 hrs.

Fig 6.5

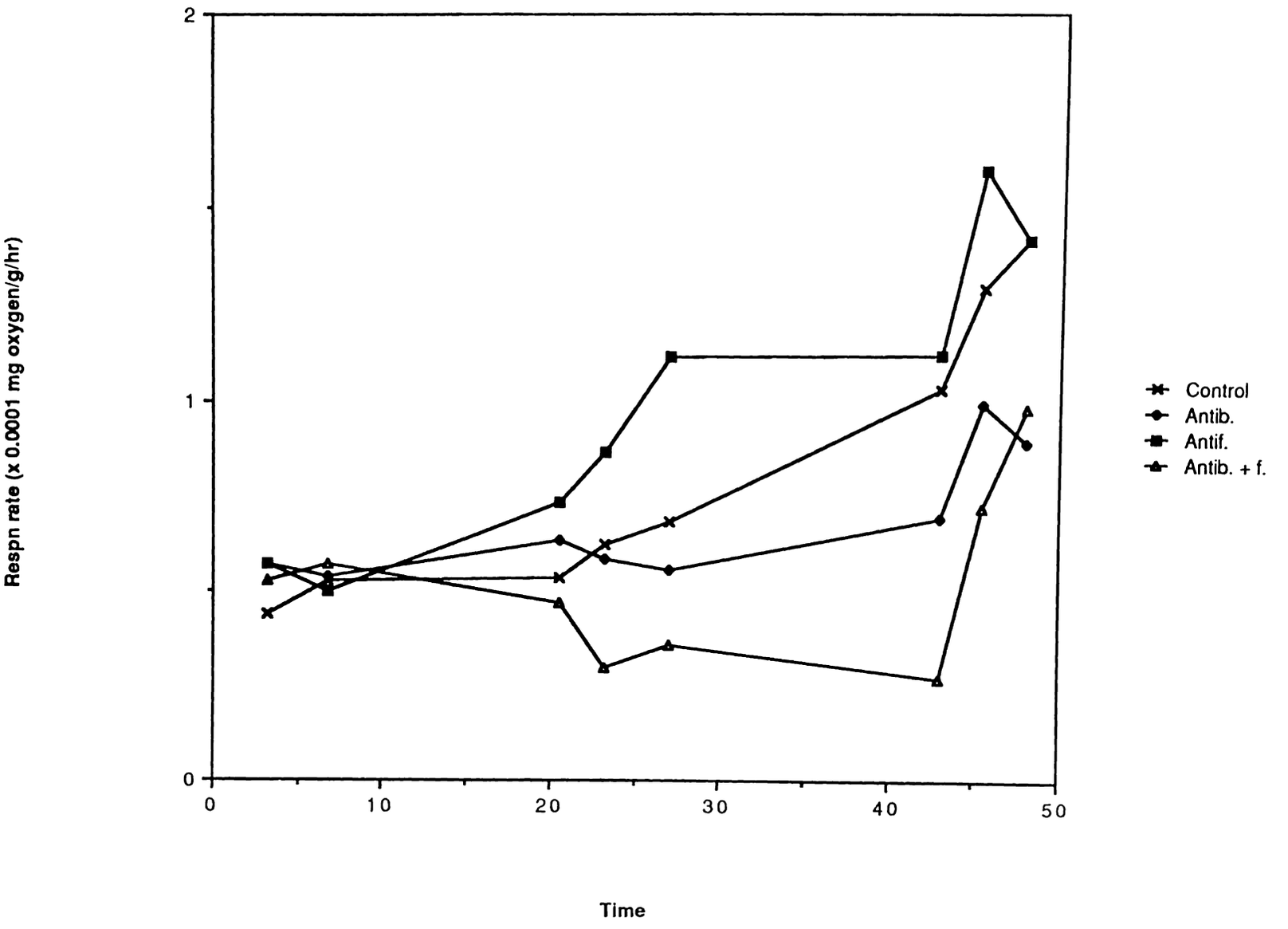
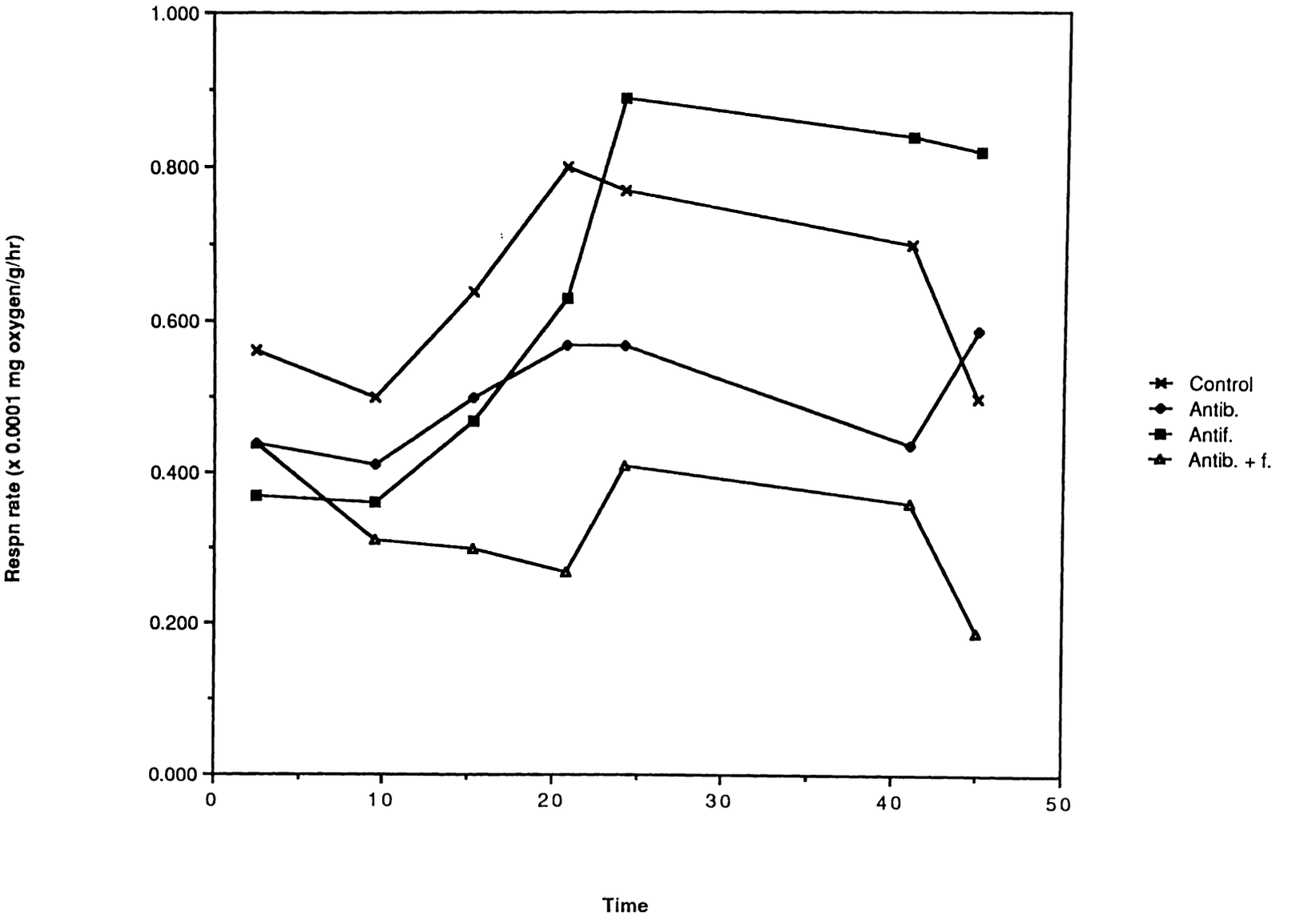

Respn rate (x 0.0001 mg oxygen/g/hr)
2
1
0
0
10
20
30
40
50
Time
Control
Antib.
Antif.
Antib. + f.

Fig. 6.6: Respiration rate of randomly collected leaves from Reid's Ck (24/11/86), following the addition of either complete nutrient solution alone (control), or complete nutrient solution with 50 ppm chloramphenicol (antib. 50), 50 ppm cycloheximide plus 50 ppm nystatin (antif. 50), or 50 ppm chloramphenicol plus 50 ppm cycloheximide plus 50 ppm nystatin (antib.+f. 50). Each treatment was replicated three times and the average graphed here. The nutrient/antibiotic solutions were not replaced in this experiment.

Fig 6.6


1.000
0.800
0.600
0.400
0.200
0.000
0
10
20
30
40
50
Respn rate (x 0.0001 mg oxygen/g/hr)
Time
Control
Antib.
Antif.
Antib. + f.

reverse inhibition, at least in the short period encompassed by the S.I.R. experiments.

The relative proportions of the total stimulated respiration attributable to either bacteria or fungi could be calculated wherever the control respiration was clearly greater than the antibacterial + antifungal treated respiration together with one or other of the other treatments. Where respiration of antibacterial and of antifungal treatments were both lower than the control, two estimates of the fungal fraction (as % of total respiration) were possible:

$$1)\ \frac{\text{control minus antifungal respiration} \times 100}{\text{control minus (antibacterial + antifungal) respiration}}$$

$$2)\ 100 - \frac{\text{control minus antibacterial respiration} \times 100}{\text{control minus (antibacterial + antifungal) respiration}}$$

If the effects of the separate antibiotics were additive the two estimates should be equal. This could be tested only in the first 12 hours of the experiments, before the respiration of the antifungal bottles surpassed that of the controls, but in general additivity was good in this period.

In Table 6.3 the two estimates have been combined (where both were calculable), together with the different antibiotic concentrations where there were more than one, to give average values of the proportion of total microbial respiration attributable to bacteria and to fungi at different times during the experiment. An estimate of the initial proportions was then made from these values.

Table 6.3: Proportion of total microbial respiration attributable to bacteria or fungi in each of five litter collections, measured periodically over up to 68 hours, followed by the estimate from these of the bacterial and fungal fractions at time = 0 hours.

	Time				Estimate
Hakarimata 28/4/86					
	10 hours	21 hours	42 hours	68 hours	
Bacteria	51 %	46 %	52 %	52 %	50 %
Fungi	50 %	57 %	48 %	54 %	52 %
Hakarimata 16/6/86					
	22 hours	25 hours	31 hours	48 hours	
Bacteria	59 %	50 %	51 %	62 %	53 %
Fungi	41 %	50 %	49 %	38 %	47 %
Hakarimata 19/11/86					
	6 hours	22 hours	26 hours	27 hours	
Bacteria	53 %	76 %	59 %	100 %	53 %
Fungi	47 %	24 %	41 %	0 %	47 %
Hakarimata 2/12/86					
	22 hrs	26 hrs	30 hrs	45 hrs	51 hrs	
Bacteria	17 %	39 %	44 %	52 %	83 %	15 %
Fungi	83 %	61 %	56 %	48 %	17 %	85 %
Reid's Ck 24/11/86					
	10 hours	15 hours	21 hours	24 hours	
Bacteria	46 %	42 %	53 %	56 %	49 %
Fungi	66 %	54 %	40 %	44 %	51 %

In three cases there was a period, usually before 48 hours, during which the ratio between the bacterial and fungal respiration calculated at each time remained fairly constant between the sampling times. In these cases the estimated in-stream (time = 0)

fractions were obtained simply by averaging the values calculated at different times. In the December sample the fungal contribution to respiration decreased steadily with time. Thus the in-stream estimate was obtained by extending the pattern back, assuming that the decrease was due to a change in composition of the microbial population, not a difference in the rate of action of the antibiotics (as there was no sign of this in other samples). In the November sample from Hakarimata there was good correspondence between the two estimates of the fungal fraction in the 6 hour measurement, but later estimates, when only the second method of calculation could be used (see Fig. 6.4), showed very high variability. Thus the first estimate was taken as the best measurement of fungal importance.

In conclusion, fungi were consistently responsible for about 50 % of the total microbial respiration, with one exception, that being the December sample from Hakarimata (85 %). This may represent the variation which can occur between samples, or may be an anomalous figure (it was notable that this was the only sample which showed a steady directional change in the importance of fungi during incubation).

Aquatic hyphomycete spore production was used to subdivide the fungal fraction of microbial respiration into that attributable to aquatic hyphomycetes and that due to other fungi. Aquatic hyphomycete spore production was measured from duplicate samples of the leaf material used in the S.I.R. experiments detailed above, except for the June sample. By multiplying the spore production figures by the respiration/spore figures obtained earlier (4.2.6 and 4.2.7), the respiration rate which would be expected from the sporulating aquatic hyphomycetes can be calculated. To do this the spore production figures were divided into

1)*L. cymbiformis* or species with similar respiration/spore values, and

2)*T. chaetocladium* or similar species (assuming that the mycelium/spore values are a reasonable indication of the respiration/spore values where the latter were not measured). The initial respiration rate recorded from the leaf samples can be found from Figs 6.2 -6.6 and the fraction of this total respiration estimated above to be due to fungi can be subdivided into that due to aquatic hyphomycetes (calculated from sporulation), and that due to other fungi (fungal respiration minus that calculated from sporulation to be due to aquatic hyphomycetes). Each step of this calculation is shown in Table 6.4 below.

Table 6.4: Spore production (divided into 1)*L. cymbiformis* and species with similar respiration/spore values, and 2)*T. chaetocladium* and species with similar respiration/spore values), followed by the respiration rate expected from the sporulating fungi (in total), then the actual rate observed from each sample. In the final line the % fraction of total fungal respiration attributable to aquatic hyphomycetes is given.

	Sample			
	Hakarimata 28/4/86	Hakarimata 19/11/86	Hakarimata 2/12/86	Reid's Ck 24/11/86
Spores/g 1)	3.9 x 106	3.6 x 106	4.5 x 106	8.5 x 104
2)	0.2 x 106	0.4 x 106	0.2 x 106	3.8 x 104
Expected Respiration (aquatic hyphomycetes)				
mg O_2/g/h	0.17	0.19	0.19	0.009
Observed Respiration (total fungi)				
mg O_2/g/h	0.21	0.14	0.30	0.11
A.H./Total	80 %	130 %	62 %	8 %

The respiration/spore figures used in these calculations were those from cultures on malt agar-(4.2.6). Similar results could be obtained using the figures from the sterile leaf experiment (4.2.7), provided the figure for *T. chaetocladium* on beech was omitted, this figure being inexplicably greater than that from kamahi. The level of variation between the remaining respiration/spore figures is important to the calculations above. In *L. cymbiformis* the mean respiration/spore figure was 2.4×10^{-8} mg O_2/g/hr, the standard deviation 1.3×10^{-8} (4.2.7). The expected respiration values calculated will have a similar level of variation within them (i.e. standard deviation of about 50 % of their value). Thus it is difficult to draw conclusions about the fraction of total fungal respiration belonging to aquatic hyphomycetes without a large number of samples. However, with the exception of the sample from Reid's Ck, aquatic hyphomycetes consistantly comprised well over 50 % of the total fungal respiration. Thus it seems likely that aquatic hyphomycetes are the most abundant group of fungi on leaves in Hakarimata.

In future work using sporulation to estimate aquatic hyphomycete respiration it is suggested that sporulation should be measured daily for four days to ensure measuring the peak, rather than just on day 2 as was done in the present work. Sporulation either side of the actual peak rate may be half the peak rate, thus reducing by half the apparent contribution by aquatic hyphomycetes to fungal respiration.

The importance of aquatic hyphomycetes in Reid's Ck appeared to be markedly different from that in Hakarimata. The sporulation figure for the Reid's Ck sample was typical of those found regularly in the leaf bag experiment, that is about ten times lower than that typically measured in Hakarimata. However, although *Lunulospora*

spp. and *T. chaetocladium* -like species dominated the spore production, as in Hakarimata, *Alatospora* -like species, although present in smaller numbers, dominated the aquatic hyphomycete mycelial content of the Reid's Ck sample, due to their high mycelium/spore values. The respiration/spore ratios for these species are not known, but are likely to proportional to their mycelium/spore values. If this is the case the aquatic hyphomycete respiration calculated for Reid's Ck will be a considerable underestimate. Further work, measuring the vegetative respiration/maximum spore production ratios for *Alatospora* and similar species would be required to check this. It is also possible the total sample respiration rate had already increased from the in-stream rate during the longer lag between collection and the first measurement of respiration for Reid's Ck samples. If the same effect was occurring in the Hakarimata samples, though to a lesser degree in the shorter time span between collection and measurement, the importance of aquatic hyphomycetes may have been slightly underestimated in this stream also.

The method of calculating the fraction of fungal respiration attributable to aquatic hyphomycetes should have provided a rough check that the fungal contribution to total respiration is not greatly underestimated, in that the respiration attributable to aquatic hyphomycetes should not be greater than that attributed to fungi in total. The fact that the expected respiration rates were not significantly greater than the observed rates in three out of four samples indicates that the S.I.R. technique does not routinely underestimate the importance of fungal respiration, although the fungal fraction of the November sample appears to have been noticeably underestimated. If the technique overestimates the

importance of fungi it could only be by the amount of respiration not accounted for by aquatic hyphomycetes, that is by considerably less than half the total fungal respiration. A more accurate test of the technique than this cannot be obtained from the present experimental results.

6.2 FREQUENCY OF DETECTION OF AQUATIC HYPHOMYCETES BY PARTICLE PLATING.

There were two aims in this section of the work. The first was to measure the frequency of detection, by plating, of aquatic hyphomycetes compared to that of fungi other than aquatic hyphomycetes. The second was to measure the frequency of detection of different aquatic hyphomycete species in order to compare their relative importance with that obtained from sporulation measurements.

In this work leaf material from the leaf bag experiment in Hakarimata and Reid's Ck was plated during July and December 1983, when both first and second run leaves (originally introduced into the streams in December 1982 and May 1983 respectively) were present. One hundred discs of each leaf type were washed, and if sufficiently entire structurally, a further hundred were surface sterilized, prior to plating out at 5 discs per Petri dish (see methods, 2.2.4). The fungal flora of approximately half of these discs were examined in detail (6.2.2).

6.2.1 Sporangial fungi.

In July a visual assessment of all discs one week after plating showed large, rapidly growing fungal colonies which probably belonged to pythiaceous species. Frequent sightings of sporangia when the colonies were submerged later to identify the aquatic hyphomycetes present confirmed this but were not quantified on this occasion. However, one fast growing colony type was later found to belong to a *Cylindrocarpon* sp., so the results may be an overestimate of the presence of pythiaceous fungi. The % frequency of discs with large, apparently pythiaceous colonies growing from them are given in Table 6.5.

Table 6.5: Percentage frequency of fresh or fallen (senescent) kamahi or beech leaf discs (n = 100) either washed or surface sterilised and plated on malt agar in July 1983 which gave rise to large, probably pythiaceous colonies. First run leaves had been immersed in the streams for 7 months prior to plating, the second run leaves for two months prior to plating.

Leaf Type and Run	% Frequency
REID'S CK	
Kamahi 1 (washed)	98
Beech 1 (washed)	99
Beech 1 (surface sterilised)	24
Kamahi 2 (washed)	74
Kamahi 2 (surface sterilised)	46
Beech 2 (washed)	92
Beech 2 (surface sterilised)	76
Fallen beech 2 (washed)	58
Fallen beech 2 (surface sterilised)	23

Table 6.5 cont.

Leaf Type and Run	% Frequency
HAKARIMATA	
(Washed leaf plates all overgrown after one week.)	
Kamahi 2 (surface sterilised)	81
Fallen kamahi 2 (surface sterilised)	83
Beech 2 (surface sterilised)	92

In December the plating operations were repeated in more detail. The mycelial types growing from each disc were described and a representative sample of each was subcultured to confirm its identity as a conidial, pythiaceous, or other fungal type. In order to check that the fungi were not over-growing the leaves in the day long interval between collection and plating a subsample of the leaves from Hakarimata was surface sterilised and plated on the day of collection. The % frequency of discs of all leaf types with large, apparently pythiaceous colony types growing from them are given in table 6.6.

Table 6.6: Percentage frequency of kamahi and beech leaf discs (n = 100) either washed or surface sterilised and plated on malt agar in December 1983 which gave rise to large, probably pythiaceous colonies. First run leaves had been immersed in the streams for 12 months prior to plating, second run leaves for 7 months prior to plating. One set of kamahi discs was surface sterilised and plated on the day of collection (day 1), the rest on the following day.

Table 6.6 cont.

Leaf Type and Run	% Frequency
REID'S CK	
Kamahi 1 (washed)	88
Beech 1 (washed)	80
Kamahi 2 (washed)	62
Beech 2 (washed)	71
Beech 2 (surface sterilised)	77
HAKARIMATA	
Kamahi 2 (washed)	85
Kamahi 2 (surface sterilised day 1)	68
Kamahi 2 (surface sterilised)	74
Beech 2 (washed)	81

In addition, in December records were kept of the occurrence of sporangia (*Pythium* or *Phytophthora* spp.), vesicles, oogonia, or zoospores seen when the agar surrounding the leaf discs was submerged to identify the aquatic hyphomycetes present. As this was 4 weeks or more after the discs were plated it was likely some contamination at least would have occurred between discs on the same plates. For this reason the number of plates out of 10 in which sporangia etc were seen on any of the five discs, as well as the % frequency of individual discs with them were counted (Table 6.7).

Table 6.7: Number of plates, and percentage frequency of kamahi and beech leaf discs (n = 50), either washed or surface sterilised, plated on them in December 1983, which gave rise to pythiaceous structures (zoospores, sporangia, vesicles, or oogonia). First run leaves had been immersed in the streams for 12 months prior to plating, and second run leaves for 7 months prior to plating. One set of kamahi discs was surface sterilised and plated on the day of collection (day 1), the rest on the following day.

Leaf Type and Run	No. plates	% Frequency
REID'S CK		
Kamahi 1 (washed)	10	30
Beech 1 (washed)	10	40
Kamahi 2 (washed)	7	30
Beech 2 (washed)	8	30
Beech 2 (surface sterilised)	9	20+
HAKARIMATA		
Kamahi 2 (washed)	10	90
Kamahi 2 (surface sterilised day 1)	10	76
Kamahi 2 (surface sterilised)	10	78
Beech 2 (washed)	10	84

Surface sterilisation appeared to cause a reduction in the frequency of detection of pythiaceous fungi, visible from the records of large colony types (Table 6.5 and 6.6) and of microscopic structures (Table 6.7). There was no parallel reduction in the number of discs giving rise to small colonies, with the exception of first run beech (Reid's Ck, July). This suggests that the sterilisation treatment did not penetrate the leaves except in this

type. Thus most, though not all, of the pythiaceous fungi present appeared to have colonised the leaf interior of both beech and kamahi. It is clear from the present results that pythiaceous fungi were a normal and abundant part of the leaf mycoflora in Reid's Ck and Hakarimata. Their frequency of detection matched that of the most abundant aquatic hyphomycete species.

There tended to be more large colonies growing from first run compared to second run leaf discs (Table 6.5 and 6.6), suggesting colonisation increased during decay. A similar pattern appeared in the records of microscopic structures although these were very limited. The records of microscopic structures (Table 5.7) showed a marked difference between the two streams, with pythiaceous fungi apparently being more abundant in Hakarimata. No such difference appeared in the colony records. If colonisation does increase during decay a difference would be expected as there was a considerable difference in decay rate in the two streams over winter. There was no difference in the frequency of detection of pythiaceous fungi between the two leaf types.

The frequencies of occurrence of large colony types (Table 6.6) were greater than those from observation of microscopic structures (Table 6.7) in Reid's Ck, but were not significantly different in Hakarimata. Either there was a large number of very fast growing fungi other than pythiaceous types in Reid's Ck which did not occur in Hakarimata, or some component of the Hakarimata bacterial microflora encouraged abundant sporulation (the main criterion for observation), or the pythiaceous flora of the two streams differed in sporulating compared with nonsporulating species or varieties.

Some zygomycetes were seen in December, but rarely: on 4 % of discs of first run kamahi and on 2 % of first run beech, both in Reid's Ck.

6.2.2 Conidial fungi.

Most of the conidial fungi present, including the aquatic hyphomycetes, could not be distinguished on the basis of colony characteristics. In addition, aquatic hyphomycetes do not normally sporulate unless submerged in water. Thus in order to identify and enumerate the conidial fungi, including aquatic hyphomycetes, it was necessary to submerge the cultures and then to examine them microscopically.

In the July plating experiment agar from under and around the first run kamahi (washed), first run beech (washed and surface sterilised), second run kamahi (washed), and second run beech (washed) discs from Reid's Ck was submerged and examined using a stereo microscope only to enumerate the conidial fungi. First run kamahi (washed), second run kamahi (washed and surface sterilised), and second run beech (washed) from Hakarimata were similarly examined using a stereo microscope, in addition to which slides of agar from most discs of second run kamahi (washed, Hakarimata) were made, to enumerate the smaller-spored fungi, which were more difficult to detect or identify under stereo microscope. In particular, *C. longibrachiata* and *C. stellata* were rarely seen with the stereo microscope, and several other species with small tetraradiate spores (*A. acuminata*, *A. pulchella*, *T. marchalianum*) were difficult to distinguish. *A. tetracladia* and *F. eccentrica* were hard to separate, likewise *L. aquatica* and *T. elegans*, and occasionally *T. chaetocladium* where there were only a few spores. All the large amerospore types (*E. microaquatica*, Unidentified spp. 1, and 2) were indistinguishable from *L. ovalis* sp. ined. and *D.*

foliicola at low power. Many of the rod-shaped spores, including *H. lugdunensis*, were also indistinguishable from each other.

In the December plating experiment one or two slides were made from submerged agar pieces taken from about the discs, for all leaf types, in addition to the stereo microscope examination. Only aquatic hyphomycete and similar large spore types were counted separately in both months. Very small conidial types (amerospores and didymospores) were counted together as a single unidentified group.

The results for the identified species for both months are given in Tables 6.9 -6.12 as frequency of discs on which recorded, and in Tables 6.13 and 6.13a as % of total species composition. The dominant species (species comprising at least 10 % of the total species composition on at least one leaf type) were similar to those found by the sporulation technique.

The frequency of detection of unidentified small conidial types is given in Table 6.8. On only one occasion was there a difference between washed and surface sterilised leaves (kamahi in Hakarimata in July), with surface sterilising apparently reducing the frequency of isolation of these small spored species. Thus in most cases these fungi had invaded the leaf interior, and were a natural part of the active fungal flora. While they were not as abundant as the major aquatic hyphomycete species recorded in the present work, conidial fungi other than aquatic hyphomycetes were present in significant numbers in the leaves.

Combining the figures for frequency of detection of pythiaceous fungi (Table 6.5, 6.6, and 6.7), with those of unidentified small conidial types (Table 6.8) and the unidentified spore types in Tables 6.9 -6.12, it is clear that from the plating data fungi other

Table 6.8: Frequency of detection of unidentified small conidial types (small amerospores and didymospores) from discs of different leaf types from Hakarimata and Reid's Ck plated onto malt agar in July and December (1983), with or without surface sterilisation. In the December plating one sample was surface sterilised and plated immediately (day 1) and the remaining samples on the following day. Results are given as frequency out of ten plates and as frequency per disc (at 5 discs per plate = out of 50 discs). The leaf types used were kamahi and beech, both first run (introduced to the streams in December 1982) and second run (May 1983) leaves.

Leaf type	No. of plates	No. discs with 1 spore type	No. discs with 2 spore types
JULY/REID'S CK			
Kamahi 1 (washed):		15/50	
Beech 1 (washed):		3/50	
Beech 1 (s. st):		5/50	
Kamahi 2 (washed):		26/50	
Beech 2 (washed):		4/50	
JULY/HAKARIMATA			
Kamahi 1 (washed):		27/50	

Table 6.8 cont.

Leaf type	No. of plates	No. discs with 1 spore type	No. discs with 2 spore types
Kamahi 2 (washed):		13/50	7/50
Kamahi 2 (s. st.):		11/50	
Beech 2 (washed):		2	
DECEMBER/REID'S CK			
Kamahi 1 (washed):	8/10	20/50	2/50
Beech 1 (washed):	10/10	27/50	1/50
Kamahi 2 (washed):	5/10	8/50	0/50
Beech 2 (washed):	5/10	11/50	0/50
Beech 2 (s. st.):	3/10	3/50	0/50
DECEMBER/HAKARIMATA			
Kamahi 2 (washed):	10/10	19/50	4/50
Kamahi 2 (s.st. day 1):	10/10	21/50	1/50
Kamahi 2 (s.st. day 2):	10/10	19/50	4/50
Beech 2 (washed):	10/10	20/50	3/50

Table 6.9: Frequency of detection of aquatic hyphomycetes from discs of different leaf types from the Hakarimata stream plated onto malt agar in July 1983, with or without surface sterilisation. Results are given as total number of species per disc, followed by the frequency of occurrence out of 100 discs for each species. The leaf types used were kamahi and beech, both first run (introduced to the streams in December 1982) and second run (May 1983) leaves.

	Leaf Types			
	Kamahi 1 (washed)	Kamahi 2 (washed)	Kamahi 2 (s. st.)	Beech 2 (washed)
No. of spp/disc:	1.1	2.5	1.1	2.0
Alatospora sp.		2		
A. filiformis		6		
A. longissima	60	2	2	8
A. tetracladia		2		
C. aquatica	8	6		16
E. microaquatica		10		
F. eccentrica		2		
H. lugdunensis		16		
L. aquatica			2	
L. ovalis		32	16	66
(L. ovalis + D. fol.)		30		
Lunulospora spp.		12	6	18
T. marchalianum		4		2
T. chaetocladium		48	38	58
T. splendens	6	2	2	
Tricladium sp.	2			

Table 6.9

	Leaf Types			
	Kamahi 1 (washed)	Kamahi 2 (washed)	Kamahi 2 (s. st.)	Beech 2 (washed)
Unident. sp. 1				
Unident. sp. 2				
Unident. s/r 2		4		
Unident. s/r 4	30	16	20	6
unident. tetrarad		2	4	12
unident. sigmoid	2	14	10	18

Table 6.10: Frequency of detection of aquatic hyphomycetes from discs of different leaf types from Reid's Ck plated onto malt agar in July 1983, with or without surface sterilisation. Results are given as total number of species per disc, followed by the frequency of occurrence out of 100 discs for each species. The leaf types used were kamahi and beech, both first run (introduced to the streams in December 1982) and second run (May 1983) leaves.

	Leaf Types				
	Kam. 1 washed	Beech 1 washed	Beech 1 s.st.	Kam. 2 washed	Beech 2 washed
No. spp/disc:	1.1	3.3	1.2	1.1	2.5
A. acuminata	(counted in unident. tetrarad.)				
A. filiformis	18	68	28	16	48
A. longissima		2			
A. tetracladia	6	22	4	42	34
C. aquatica	8	60	13		6
Culic. aquatica					2
Filosporella sp.		2	2		
H. lugdunensis	18	18	34	10	24
L. aquatica		5			12
Lunulospora spp.	12	57	11	2	20
T. elegans	2	10		14	50
T. chaetocladium	18	27	4	6	20
T. splendens		3		2	
Unident. s/r 2		2			
Unident. s/r 4	2				
unident. tetrarad	4	25	6	14	12
unident. sigmoid	22	25	13	6	10

Table 6.11: Frequency of detection of aquatic hyphomycetes from discs of different leaf types from the Hakarimata stream plated onto malt agar in December 1983, with or without surface sterilisation. One sample was surface sterilised and plated immediately (day 1) and the remaining samples on the following day. Results are given as total number of species per disc, followed by the frequency of occurrence out of 100 discs for each species. The leaf types used were second run kamahi and beech (introduced into the streams in May 1983).

	Leaf Types			
	Kamahi washed	Kamahi s.st. 1	Kamahi s.st. 2	Beech washed
No. spp/disc:	2.8	1.9	2.4	2.6
A. acuminata			2	2
Alatospora sp.	10	4		
A. crassa	4		4	6
A. filiformis	16	22	18	
A. longissima	4	2	2	6
A. tetracladia	4	4	2	
C. aquatica	6		14	14
D. foliicola		2		12
E. microaquatica	10	10	16	12
Filosporella sp.	2	6	2	2
F. penicillioides	4		2	8
H. lugdunensis			2	2
L. ovalis	8	24	8	2
Lunulospora spp.	4	10		14
T. marchalianum	36	16	14	26

Table 6.11 cont.

	Leaf Types			
	Kamahi washed	Kamahi s.st. 1	Kamahi s.st. 2	Beech washed
T. setigerum	2			
T. chaetocladium	18	12	18	30
T. splendens	4		2	10
Tricladium sp.	2		2	
T. myrti	2			
Unident. sp. 1	6	2	8	40
Unident. s/r 1(3)	2		2	
Unident. s/r 2	8	4	12	6
Unident. s/r 4	68	60	65	34
Unident. s/r 6	8		6	12
Unident. s/r 7	4			4
Unident. s/r 8				2
Unident. s/r 9	2	12	4	2
Unident. s/r 10			4	
Unident. s/r 12	36	4	22	8
Unident. s/r 13	2			
Unident. s/r 18			6	
Unident. sigmoids	2		2	2

Table 6.12: Frequency of detection of aquatic hyphomycetes from discs of different leaf types from Reid's Ck plated onto malt agar in December 1983, with or without surface sterilisation. Results are given as total number of species per disc, followed by the frequency of occurrence out of 100 discs for each species. The leaf types used were kamahi and beech, both first run (introduced to the streams in December 1982) and second run (May 1983) leaves.

	Leaf Types				
	Kam. 1 washed	Beech 1 washed	Kam. 2 washed	Beech 2 washed	Beech 2 s.st.
No. spp/disc:	1.6	3.3	4.6	4.9	4.3
A. acuminata		12	16	24	10
A. crassa		2	2		2
A. filiformis	4	82	90	90	80
A. tetracladia	12	52	12	66	64
C. aquatica		2	20	24	36
C. longibrachiata				2	
D. aquatica			2	2	10
Filosporella sp.	4	4		2	
F. penicillioides	2	2			
F. eccentrica		8	94	14	16
H. lugdunensis	26	66	42	50	62
L. aquatica	2		4		
Lunulospora spp.		14	10	24	18
M. acerina					2
T. elegans		14	48	28	40
T. setigerum	4			2	

Table 6.12 cont.

	Leaf Types				
	Kam. 1 washed	Beech 1 washed	Kam. 2 washed	Beech 2 washed	Beech 2 s.st.
T. chaetocladium	20	30	18	64	36
V. elodeae	2		8		
Unident. sp. 1	2				
Unident. sp. 2(?)			68	50	28
Unident. s/r 1(3)		2	2	8	6
Unident. s/r 2	34	2		4	6
Unident. s/r 3		4	2		2
Unident. s/r 4	16	4	4		2
Unident. s/r 6	10	26	4	2	2
Unident. s/r 7	4	2			
Unident. s/r 8	8		4		
Unident. s/r 11	6	2	2	2	
Unident. s/r 12	2		2	2	2
Unident. s/r 14		2	4	2	
Unident. s/r 18				20	6
unident. sigmoid			2		2

than aquatic hyphomycetes were almost as abundant as the aquatic hyphomycetes themselves. Thus aquatic hyphomycetes do not dominate the mycoflora, as measured by frequency of detection by particle plating, as clearly as they appeared to when calculated above from the S.I.R. work (6.1.3).

In Table 6.13 and 6.13a the aquatic hyphomycete flora of the washed plated discs for December and July, as % composition, derived from frequency of detection, is compared with that derived for the same leaf samples from spore production and from mycelial content. It was expected that the frequency of detection by plating would correspond more closely to the relative mycelial fractions of each species than to the spore counts. Thus the plating technique could provide a final test of the sporulation method of measuring the mycelial content of leaves. Obviously, only species for which mycelium/spore conversion factors had been measured could be compared to test this, and only when the leaf sample was dominated by these species should % composition figures from mycelial content be comparable to those from frequency of detection by plating. Thus first run kamahi in Reid's Ck (December) was of little use.

The number of species detected by the sporulation and plating techniques differed. The number of species on second run kamahi and beech in Reid's Ck in the sporulation measurements increased from July to December but all other leaf types in Reid's Ck and all types in Hakarimata decreased. The number of species found from plating increased markedly on all leaves, probably as a result of the increased observation. Thus there were many more species detected by plating than spore counts in December, particularly on the first run leaves, which did not show the conspicuously reduced floras compared with second run leaves visible from the sporulation measurements.

Table 6.13: Percent composition of aquatic hyphomycete flora on the fresh kamahi and beech leaf types used in the leaf bag experiment, as determined from plating frequencies (1), spore numbers (2), and the mycelial content (3) calculated from spore production, all measured from the same material (collected in July, 1983). First run leaves (introduced initially in December 1982) and second run leaves (introduced initially in May 1983) were measured on both occasions, except when one or more measurements could not be made, either because of insufficient material remaining, or dominated by fungi other than aquatic hyphomycetes. Where spore production was recorded but no mycelium/spore conversion factors was available, the % mycelial content is marked "?". Where two sets of plating frequencies were obtained, from washed and from surface sterilised leaves of the same type, an average % of these is given.

	Hakarimata/July									Reid's Ck/July											
	Kamahi 1			Kamahi 2			Beech 2			Kamahi 1			Beech 1			Kamahi 2			Beech 2		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
A. acuminata											6.8	25.6		3.9	33.1		1.0	7.4			
Alatospora sp.		0.2	0.3	0.5	1.4	28.5		0.2	6.2		0.6	2.2		1.4	11.9						
A. crassa+longiss.	56.0	31.3	41.3	1.5	0.1	1.2	4.0				0.8	3.1	0.5	0.2	1.4						
A. filiformis				4.5	0.2	0.3		<0.1	0.2	16	1.4	0.3	22.5	2.9	1.5	14.0	39.4	17.9	19.0	4.8	0.9
A. tetracladia				1.0						5.0	2.2	0.4	5.0	0.8	0.3	37.0	6.4	2.4	14.0	0.5	0.1
C. aquatica	7.0	5.2	0.7	1.0	0.5	1.1	8.0	1.0	2.9	8.0	36.1	13.6	14.5	32.4	27.3				2.0	0.5	0.1
C. longibrachiata		44.0	57.3		0.4	8.8		0.6	18.0		12.6	47.3					2.3	16.5		28.4	79.7
Culic. aquatica											0.1	?					0.2	?	1.0		

Table 6.13 cont.

	Hakarimata/July									Reid's Ck/July									
	Kamahi 1			Kamahi 2			Beech 2			Kamahi 1			Beech 1			Kamahi 2			Beech 2		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
D. aquatica														0.01							
E. microaquatica		0.2	?	5.5	1.1	?		0.2	?												
Filosporella sp.											2.2	0.2	1.5				0.2	<0.1		3.6	0.3
F. penicillioides					0.1	<0.1		<0.1	<0.1												
F. eccentrica				0.5							0.01					1.0	0.7	0.3			
H. stellatacula		0.41	?								5.3						5.0			3.6	
H. lugdunensis				3.0						16.0			17.5			9.0	0.5	?	9.0	2.1	?
L. aquatica				1.0	0.2	0.1					0.2	<0.1	1.5				3.7	1.4	5.0		
L. ovalis		3.2	0.1	14.0	50.3	20.1	32.0	64.6	36.0												
(L. ovalis + D. fol.)				6.0																	
Lunulospora spp.		0.2	<0.1	5.5	30.7	12.2	9.0	16.7	9.3	11.0	10.8	0.7	13.5	51.9	8.8	1.0	1.5	0.2	8.0		
T. gracilis														<0.1							
T. elegans					<0.1	<0.1				2.0			1.5	0.1	0.2	12.0	36.2	52.2	20.0	28.2	15.8
T. marchalianum				1.0	<0.1	<0.1		1.0													
T. chaetocladium		3.3	0.9	27.0	6.1	24.2	28.0	4.8	26.9	16.0	2.2	1.7	5.5	0.4	0.8	5.0	0.5	0.8	80		
T. splendens	6.0			1.5									0.5	0.16	?	1.0					

Table 6.13 cont.

	Hakarimata/July									Reid's Ck/July									
	Kamahi 1			Kamahi 2			Beech 2			Kamahi 1			Beech 1			Kamahi 2			Beech 2		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Tricladium sp.	2.0																				
T. acuminatus		0.6	?								0.7?	0.3	?								
V. elodeae																			2.0		
Unident. s/r 1(2)					0.1	<0.1					0.3	<0.1		0.7	<0.1		1.5	<0.1			
Unident. s/r 1(3)											1.1	4.0	0.5	1.7	14.1					0.5	1.4
Unident. s/r 2				1.0									0.5								
Unident. s/r 4	28.0			17.5	0.91	?	3.0			2.0											
Unident. s/r 10								<0.1	?		0.2	?									
Unident. s/r 12		0.4	?		6.6	?		0.1	?		3.9	?		1.3	?					23.3	?
Unident. s/r 14					0.1	?															
Unident. s/r 16		9.5	?					1.2	?												
Unident. s/r 18		1.2	?								0.1	?			0.4	?					
unident. tetrarad.				2.5			3.0				0.2	?	6.5	0.3	?	12.0	0.8	?	7.0	3.6	?
unident. sigmoid	2.0	<0.1	?	7.5	1.1	?	9.0			19.0	0.6	?	9.5			5.0			4.0		
unident. spores														11.6			1.0				

Table 6.13a: Percent composition of aquatic hyphomycete flora on the fresh kamahi and beech leaf types used in the leaf bag experiment, as determined from plating frequencies (1), spore numbers (2), and the mycelial content (3) calculated from spore production, all measured from the same material (collected in December, 1983). First run leaves (introduced initially in December 1982) and second run leaves (introduced initially in May 1983) were measured on both occasions, except when one or more measurements could not be made, either because of insufficient material remaining, or material dominated by fungi other than aquatic hyphomycetes. Where spore production was recorded but no mycelium/spore conversion factor was available, the % mycelial content is marked "?". Where two sets of plating frequencies were obtained, from washed and from surface sterilised leaves of the same type, an average % of these is given.

	Hakarimata/December									Reid's Ck/December								
	Kamahi 2			Beech 2			Kamahi 1			Beech 1			Kamahi 2			Beech 2		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
A. acuminata		9.5	46.4	1.0	1.59	9.4				4.0	5.6	38.9	4.0	0.6	4.1	3.5	2.4	20.0
Alatospora sp.	2.0	8.5	41.3		4.8	28.1								0.9	6.6		0.8	7.0
A. crassa+longiss.	2.0			4.0	7.9	46.8				1.0			<1.0			<1.0		
A. filiformis	8.4	0.5	0.2				3.0			25.0	5.6	2.5	20.0	24.4	10.8	18.5	9.6	5.1
A. tetracladia	1.3	0.4			0.4		8.0			16.0	5.6	2.0	3.0	4.0	1.4	15.0	0.8	0.4
C. aquatica	2.7	8.5	4.1	5.0	1.6	0.9				1.0			4.0	11.7	8.2	6.5	32.4	27.1

Table 6.13a cont.

	Hakarimata/December												Reid's Ck/December					
	Kamahi 2			Beech 2			Kamahi 1			Beech 1			Kamahi 2			Beech 2		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
C. longibrachiata														3.1	21.9	<1.0	1.8	14.8
D. aquatica													<1.0	1.1		1.1	0.1	
D. foliicola	0.3			5.0														
E. microaquatica	5.3	0.5	?	5.0														
Filosporella sp.	1.7			1.0			3.0			1.0				1.0		<1.0	0.1	<0.1
F. penicillioides	0.7			3.0			1.0			1.0								
F. eccentrica										2.0			21.0	8.7	3.1	3.5	0.8	<0.1
H. stellatacula														0.2	?			
H. lugdunensis	0.3			1.0			16.0			20.0			9.0			12.0		
L. aquatica							1.0	8.8					1.0	0.8	0.3		0.1	
L. ovalis	6.0	60.0	5.9	1.0	3.2	0.4				1.0								
Lunulospora spp.	2.0	7.4	0.7	6.0	74.6	8.8				4.0	60.5	8.4	2.0	8.9	1.2	4.5	24.1	4.0
M. acerina																<1.0		
T. gracilis												0.1	?					
T. elegans		0.4	?		0.4	?				4.0			11.0	10.8	15.1	7.5	3.1	5.2
T. marchalianum	9.0			10.0														

Table 6.13a cont.

	Hakarimata/December									Reid's Ck/December								
	Kamahi 2			Beech 2			Kamahi 1			Beech 1			Kamahi 2			Beech 2		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
T. setigerum	<0.1						3.0									<1.0		
T. chaetocladium	6.7	1.1	1.0	12.0	4.8	5.6	13.0	5.9		9.0	5.6	7.8	4.0	5.5	7.7	10.5	6.7	11.2
T. splendens	0.7			4.0														
Tricladium sp	1.0																	
T. myrti	0.3																	
V. elodeae							1.0						2.0					
Unident. sp. 1	2.0			16.0			1.0											
Unident. sp. 4								70.6										
Unident. s/r 1(2)														12.3	0.2		12.4	0.2
Unident. s/r 1(3)	0.7									1.0	5.6	38.9	<1.0	2.6	18.4	1.5	0.5	4.2
Unident. s/r 2	0.3			2.0			22.0			1.0						1.0		
Unident. s/r 3										1.0			<1.0			<1.0		
Unident. s/r 4	27.7			13.0			10.0			2.0			1.0			<1.0		
Unident. s/r 6	2.0			5.0			6.0			8.0			1.0			<1.0		
Unident. s/r 7	1.3			2.0			3.0			1.0								

Table 6.13a cont. Hakarimata/December Reid's Ck/December

	Kamahi 2			Beech 2			Kamahi 1			Beech 1			Kamahi 2			Beech 2		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Unident. s/r 8				1.0			5.0						1.0				1.74	?
Unident. s/r 9	3.0			1.0														
Unident. s/r 10	0.7				0.4	?												
Unident. s/r 11							4.0			1.0			<1.0			<1.0		
Unident. s/r 12	8.7	2.6	?	3.0			1.0	14.7	?		5.6	?	<1.0			<1.0	0.1	
Unident. s/r 13	0.3																	
Unident. s/r 14										1.0			1.0			<1.0		
Unident. s/r 18	1.0										5.6	?				2.0		
unident. tetrarad.														3.3	?		2.4	?
unident. spores	0.7	0.3	?	1.0									<1.0	0.1	?	<1.0		

In several species plating percentages (percent of total flora) were fairly consistently closer to mycelium- than sporulation-derived percentages: *L. ovalis* sp. ined., *C. aquatica*, *Lunulospora* spp., *T. chaetocladium*, and Unidentified s/r type 1(2). *A. acuminata*, *A. filiformis*, and *A. longissima/crassa* showed this pattern only once. *A. longissima/crassa* showed the reverse pattern in the only other sample in which it was abundant, that is, the plating percentage was more like that from sporulation than mycelial content. *A. filiformis* was more commonly of greater importance in the plating flora than either mycelium- or sporulation-measured floras, along with *A. tetracladia*, *H. lugdunensis*, *T. marchalianum*, *V. elodeae* (although at very low levels in the plating flora, it was not detected at all in sporulation measurements), and Unidentified s/r type 4. A large number of Unidentifed s/r types were only detected by plating, mostly at quite low levels. *A. acuminata* was more commonly comprised a smaller percent of the flora determined by the plating method than it was a fraction of the total mycelium, and to a lesser extent, total sporulation measurements, along with *A. pulchella*, *C. longibrachiata*, *H. stellata*, Unidentified s/r type 1(3), and possibly Unidentified s/r type 12.

Thus the most common species, as measured by the sporulation technique, comprised a fraction of the plating flora that corresponded better to their importance in the total mycelial content than to their percentage of total spore production. This supports the sporulation to mycelium conversion process. However, a number of important species did not. These results will be discussed in section 7.2.3.

In conclusion, the selective inhibition of respiration technique was able to show that fungi were responsible for about 50 % of total microbial respiration of naturally occurring leaves in the Hakarimata stream and Reid's Ck. The use of spore production and respiration ratios measured in Chapter 4 allowed the fungal fraction to be further subdivided into respiration attributable to aquatic hyphomycetes and that to other fungi. Aquatic hyphomycetes clearly dominated fungal respiration. The frequency of detection of fungi by particle plating techniques also indicated that aquatic hyhomycetes dominated the fungal flora, but possibly by a smaller margin than was suggested by the S.I.R. technique.

CHAPTER SEVEN: DISCUSSION OF METHODS OF MEASURING AQUATIC HYPHOMYCETE BIOMASS AND ITS RELATIVE IMPORTANCE IN LEAVES.

A major problem in microbiology is the measurement of the biomass of different taxonomic components of the microbial flora in natural substrates. In this chapter the results from Chapters Four and Six are discussed. It is concerned largely with the development, justification, and limitations of the techniques used to measure the importance and mycelial biomass of aquatic hyphomycetes in leaves in streams. The chapter is divided into two sections:

1)The selective inhibition of respiration technique;

2)Sporulation as a measure of mycelial content.

7.1 SELECTIVE INHIBITION OF RESPIRATION.

Respiration can provide a measure of microbial activity and biomass. The aim of the S.I.R. (Selective Inhibition of Respiration) technique is to partition microbial respiration into that attributable to bacteria, and that to fungi. The technique has been used before by several workers, but is not straight forward, and relies on a number of assumptions. The development and limitations of it are discussed here, followed by a comparison of the results obtained in the present study with those from previously published work.

7.1.1 Development of the selective inhibition of respiration method.

Kaushik and Hynes (1968, 1971) were probably the first to use antibiotics on leaves from a stream in order to distinguish the relative importance to leaf decay of bacteria and fungi. They incubated the leaves in stream water, modified with the antibiotics, for one month or longer and compared the protein content and weight loss of the leaves at the end of this period. The apparent fungal and bacterial contributions were not additive, indicating competition between the two groups occurs normally. Thus in a long experiment such as theirs what was obtained was a measure of the potential importance of bacteria and fungi, respectively, to decay, which was not necessarily the same as the actual contribution to decay in natural, mixed populations. Most subsequent studies, using material from streams, swamps, and soils, have measured the effect of antibiotics on microbial respiration in short term experiments to determine the bacterial and fungal fractions of microbial activity, for example, Anderson and Domsch (1973, 1974), West (1986), and West and Sparling (1986).

Kaushik and Hynes (1968, 1971) used nystatin and/or cycloheximide as antifungal antibiotics, and streptomycin and penicillin as antibacterial antibiotics. Most subsequent workers have used various combinations of these antibiotics. However, a number of oomycetous fungi are known to be sensitive to streptomycin, being affected at concentrations commonly used to inhibit bacterial respiration (Rooke and Shattock, 1983), leading to a possible overlap in antibiotic activity. Benner et al (1986) used candicidum as the antifungal antibiotic and chloramphenicol and tetracycline as the antibacterial antibiotics, in one of the few studies using other antibiotics. Padgett et al (1985) found that chloramphenicol on its own gave the greatest inhibition of bacterial

respiration in *Spartina* leaves (87 % compared with 65 % for streptomycin and penicillin together). For this reason chloramphenicol was chosen as the antibacterial antibiotic, and nystatin and cycloheximide as the antifungal antibiotics in the present study. Particle plating showed the three antibiotics to be specific to their respective target organisms, as well as highly effective at inhibiting growth and/or respiration (section 6.1.1).

Because antibiotics may become adsorbed and inactivated in soil, a considerable amount of work has been done in studies dealing with the inhibition of respiration in soil (Anderson and Domsch, 1975) to determine the optimum concentration of antibiotics to use, some soil types requiring quite high concentrations. This did not appear to be necessary in the present work as good inhibition was obtained with 50 ppm concentrations, and was not improved by increases of up to 250 ppm. Other workers, for example Padgett et al (1985), have also obtained good inhibition of respiration in leaf samples with similarly low concentrations.

In studies using stream water or other low nutrient media for incubation a large proportion of respiration frequently remained uninhibited by the presence of antibiotics. Mason (1976), for example, found 40 -50 % of respiration from reed pieces incubated in lake water remained uninhibited in his work. Even less inhibition of unmodified respiration was found in the present work. Yetka and Wiebe (1974) found that the amount of inhibition of bacteria obtained depended on the growth phase of the population. No inhibition of respiration of stationary phase bacteria occurred. The same effect might also occur with fungi. Thus in order to inhibit as much respiration as possible, and therefore to more accurately divide the total respiration into bacterial and fungal, the microorganisms in the leaves must be stimulated into an active

growth phase in which they will be susceptible to the antibiotics.

Benner et al (1986) found fungi were less important than bacteria in litter from a salt marsh when lignocellulose-stimulated respiration was measured. In comparison, Padgett et al (1985) found at least 70 % of glucose-stimulated microbial respiration of *Spartina* in a salt marsh was attributable to fungi. The difference was presumably in part due to the different substrates added. Glucose might be expected to stimulate most of the active microflora, compared with more specialised substrates, which will stimulate only that fraction of the microflora capable of using them. For this reason glucose was used as the carbon source in the present work.

Some workers have added glucose alone to stimulate respiration, while others have found the addition of other nutrients, such as N and P, to have a further stimulatory effect (Howarth and Fisher, 1976; Visser et al, 1984; Van de Werf and Verstraete, 1987a). In the present work the addition of a glucose plus complete mineral nutrient solution was found to stimulate respiration most rapidly.

7.1.2 Limitations of the selective inhibition of respiration technique.

Obviously, error would enter where there was a significant amount of respiration occurring through anaerobic pathways, as then a significant proportion of the active flora will not then be measured. However, there are several other less obvious potential sources of error in the selective inhibition of respiration technique.

The method only measures the microorganisms that respond to an influx of glucose. Lund and Goksøyr (1980) found what appeared to be two distinct groups of bacteria in soil: zymogenous bacteria, which responded directly to added nutrients, and smaller, autochthonous bacteria, which showed an indirect response. Van de Werf and Verstraete (1987b) showed that the "active" soil biomass responding to glucose comprised between 4 and 49 % of the total biomass, as determined by the fumigation -reinoculation method. The variation occurred between sites, those subject to regular organic inputs having a larger active proportion of the total biomass. This suggests that the selective inhibition of respiration technique would only partition the active rather than the total biomass present in a sample. In the case of leaf litter, however, it might be expected that there would be little difference between the active and total biomass, as the substrate in its entirety is an "organic input", unlike the soil in the study by Van de Werf and Verstraete (1987b).

Some previous workers have noted the competing or inhibitory effect of one group of microorganisms on another in the course of experiments using the selective inhibition of respiration technique. The suppression of bacteria by fungi was obvious in the present work, with the antifungal treatments showing markedly higher bacterial respiration rates than untreated controls after about 12 hours. Measurements obtained prior to this bacterial bloom were therefore valuable because the additivity of the effects of the individual antibiotics could be checked, i.e that the effect of the antibacterial antibiotic treatment added to the effect of the antifungal antibiotic treatment approximately equals the inhibition of respiration in the combined antibacterial and antifungal antibiotic treatment.

However, the preference for early sampling was limited by the length of time required by the antibiotics to achieve maximum total inhibition. As stated earlier, the more respiration that is not inhibited by either of the antibiotic groups the greater the potential error through assuming the ratio between the bacterial and fungal respiration is the same in the uninhibited as in the inhibited fraction of the respiration.

The incubation period used in previous studies has varied depending on conditions such as nutrient concentration and temperature. Mason (1976) incubated reed pieces in lake water (no added nutrients) for 36 hours. Most workers who added glucose to soil to stimulate respiration incubated material for much shorter periods: 6 -8 hours (Anderson and Domsch, 1975); a maximum of 5.5 hours (West, 1986). These later studies were carried out at 25 C, compared with 13 C in the present work, the difference in temperature probably explaining why a longer incubation was acceptable in the present work. Respiratory measurements over the first 12 hours in the present work tended to vary considerably relative to the differences between the control and antibiotic treatments, and after 48 hours often showed steady directional changes in the eukaryote:prokaryote ratio, as well as sudden, large scale, but apparently random variation, perhaps due to increased respiration from the incubation liquid itself. Thus the best respiration measurements were usually obtained between about 6 and 48 hours.

A major assumption of the S.I.R. technique is that the eukaryote:prokaryote ratio of the control material remains constant during the measurement period. However, Paul and Johnson (1977) found that bacterial numbers increased faster than fungal biomass following the addition of glucose to soil in their study. The rate

at which the different antibiotics may take effect may also affect the apparent ratio between the microbial groups at any one time. In addition to measuring respiration as early as possible after the addition of nutrients, a high frequency of measurement is important, to determine variation, and particularly directional change, in the eukaryote:prokaryote ratio. Where such variation occurs the results of the technique should be treated with great care.

The effect of the problems outlined above is to increase the amount of work required to obtain a single, reliable figure for the relative contributions to respiration of bacteria and fungi, rather than to invalidate the whole technique. It still provides virtually the only, and certainly the fastest, method of measuring the relative active biomasses of fungi and bacteria in natural substrates.

7.1.3 Comparison with previous work.

In the present work fungal respiration was estimated to comprise 47 to 85 % of the total respiration of leaves in Hakarimata stream and Reid's Ck, most commonly close to 50 %. Others have obtained similar results from both terrestrial and freshwater habitats. In temperate soils fungi comprised 50 to 90 % of total respiration of glucose (Anderson and Domsch, 1975; West, 1986). A terrestrial beech litter sample gave a figure of 60 % of total respiration of fungal origin (Anderson and Domsch, 1975). Inhibition of bacteria in experiments by Kaushik and Hynes (1968, 1971) had little effect on the decay of leaves in stream water, compared to inhibition of fungi. Triska (1970) also found that fungi in general dominated on most leaves in streams, but not on some leaf types. Mason (1976) found fungi clearly dominated the inhibited respiration of leaves in

lake water after 35 days decay, but later in decay (144 days) the position had reversed and bacteria dominated. Thus the present results correspond to an average of previous work in aquatic litter, relating to the range of leaf types and decay stages in random leaf samples.

The conspicuous abundance of aquatic hyphomycetes has, in the past, suggested that aquatic hyphomycetes were easily the most abundant fungi in stream environments (Ingold, 1976; Iqbal, 1976). In the present work the S.I.R. technique was used to measure the relative abundance of fungi, while the laboratory measurements of aquatic hyphomycete respiration and sporulation were used to divide the total fungal contribution into respiration attributable to aquatic hyphomycetes, and to other fungi (6.1.3). This method indicated 62 to approximately 100 % of fungal respiration from leaves in Hakarimata was from aquatic hyphomycetes. Thus aquatic hyphomycetes appear to be the dominant fungal group, although other fungi comprised an appreciable biomass also.

The relatively high frequencies of isolation observed in the present work suggest both conidial species other than aquatic hyphomycetes and pythiaceous fungi were important constituents of the leaf mycoflora, together probably comprising the fraction of the fungal respiration which could not be attributed to aquatic hyphomycetes, that is up to about 40 % of total fungal respiration.

Some of the non -aquatic hyphomycete conidial species identified in the present work were also reported as common in previously published work dealing with aquatic litter, for example *Fusarium* spp. (Chamier et al, 1984; Bärlocher and Kendrick, 1974; Newton, in Willoughby, 1974; Triska, 1970), and *Cylindrocarpon* spp. (Bärlocher and Kendrick, 1974). It seems likely that these fungi are a normal and active part of the leaf mycoflora worldwide, in addition to the

so-called "aquatic hyphomycetes", despite not being conspicuous in submerged sporulation measurements. Previous records have varied in the importance attributed to pythiaceous fungi. Bärlocher and Kendrick (1974) noted *Pythium* spp. "appeared sometimes" on ash, oak, and maple leaves. Triska (1970) found "fairly extensive growth" on ash and birch leaves only in the sample taken after four weeks immersion. In contrast, Newton (in Willoughby, 1974) found *Pythium* and *Phytophthora* spp. to be present throughout decay of a variety of leaf types including beech, while Chamier et al (1984) found a similar pattern of increasing then decreasing abundance to that found in aquatic hyphomycetes on alder. Thus these fungi are also probably an active part of the normal flora of decomposing leaves, possibly varying between leaf types and stage of decay.

7.2 SPORULATION AS A MEASURE OF MYCELIAL CONTENT.

Studies of aquatic hyphomycetes on leaves in streams have commonly used number of spores as a measure of relative abundance, usually with many reservations. The most general criticism is that the technique selects for prolifically sporulating species at the expense of other species of potentially greater biomass but lower sporulating capacity (Chamier et al, 1984). Bärlocher (1982a and b) emphasized that conidial counts (obtained in the laboratory) measure the reproductive potential of the fungi present, and are probably influenced by the substrate type as well as stream factors. However, these criticisms could be tested and allowance made for

differing sporulation capacities between species, and perhaps substrates. No proper assessment of the technique or of the relationship between sporulation capacity and vegetative biomass has been made prior to the present work.

Padgett (1976), in the course of a field experiment like the present leaf bag experiment, hypothesized that spore production might serve as an indicator of mycelial growth which, in turn, could be correlated with leaf disc weight loss. On finding a very low correlation between weight loss and spore production, he concluded that spore production was not an adequate index of mycelial growth. The low correlation between spore production and weight loss is hardly surprising considering the number of other sources of weight loss in a stream apart from aquatic hyphomycetes (Chapter 8 in the present work; Petersen and Cummins, 1974).

In the present work standard conditions for measuring optimum sporulation from leaves were chosen. The mycelium/spore relationship was measured under the same conditions using pure cultures of 12 species on agar. The effects on this relationship of the factors in which the pure culture conditions differed from those of leaves from streams (the presence of competing microorganisms and the biochemistry of the substrate), together with the some of the different preconditioning factors leaves could be subjected to prior to collection (nutrient concentration, oxygenation) were tested for individually.

Several other experiments were undertaken to obtain some understanding of the physiology of sporulation, and whether this was the same in fungi on agar and in naturally occurring leaves. What caused sporulation from leaves collected in streams to increase, and then to decrease? Did the same factors cause this pattern in sterile cultures? If not, were the peaks in sporulation obtained

from agar cultures and leaves comparable? This work attempted to provide a theoretical basis for the use of sporulation as a measure of biomass.

7.2.1 Mechanism of spore induction and decline in spore production of aquatic hyphomycetes in vitro and in vivo.

The normal method of obtaining spores from aquatic hyphomycetes growing on agar is to submerge pieces of the culture in deionised water. Some species of aquatic hyphomycetes on leaves collected from Hakarimata appeared able to sporulate in air (4.3.1), but no sporulation of the test species used occurred on dry agar (4.1.1, 4.1.2, and 4.2.2).

Submersion of agar cultures in deionised water could cause leaching of nutrients resulting in nutrient deprivation, reduction in the concentration of inhibitory compounds or byproducts, changes in osmotic pressure, or perhaps mechanical injury, due to cutting and washing. It was found that sporulation did not occur with nutrient depletion alone (low C agars), nor on different C sources with presumably different osmotic pressures (4.1.1 and 4.2.2, 4.2.3). While nutrient depletion did not induce sporulation without submersion, high nutrient concentrations (both C and other nutrients) could inhibit sporulation even when submerged (4.1.1). Thus nutrient concentration has an important role in the induction of sporulation in aquatic hyphomycetes, but only in conjunction with some other effect of submersion.

The liquid culture results (4.1.1) suggest that no abrupt event such as mycelial disruption, or change in osmotic pressure, was necessary for sporulation. (This assumes, in the absence of evidence to the contrary, that the induction phenomenon was the same

in pure cultures whether grown on solid media or in liquid.) The liquid culture experiment also indicated that removal of staling products was not necessary for sporulation, as sporulation increased at the same time that concentrations of staling products might be expected to be increasing. However, the low sporulation seen in the "no change" flasks suggested that in very old cultures such compounds may exist and may possibly inhibit sporulation.

Thus it is not clear how submersion of agar pieces caused the induction of sporulation, only that it did not appear to be due to nutrient deprivation alone, and probably not to removal of inhibitory compounds, changed osmotic pressure, or mechanical injury alone. However, if the induction phenomena in liquid and on solid media were not the same then the last three factors above cannot be ruled out.

What caused spore production from leaves collected from streams to increase when incubated in deionised water was even less clear. The change in nutrient concentration from stream water to deionised water would not have been sufficiently great. Possibly the disruption of the surface aufwuchs and/or fungal mycelium from the cutting and washing of the leaves was important, although cutting alone appeared to have little effect (4.3.4). A change in temperature would have occurred for some samples, but not all, whereas all samples showed an increase in sporulation. Collection of leaf litter involved removal from the water for several hours, resulting in much higher oxygen levels being available to the leaves and their microflora. Fungal sporulation is more sensitive to oxygen concentrations than vegetative growth (Turian, 1974). Whether this could have caused the increased spore production was not tested in any way in the present work.

Thus increased oxygen concentration, mycelial disruption, and

possibly temperature change were the most likely factors responsible for the increase in sporulation seen following incubation of collections of leaves from streams.

A wide variety of environmental factors have been found to induce sporulation in other fungi, such as nutrient depletion (C or N), light, partial desiccation, cutting the mycelium, high or low temperature shocks, toxins, high osmotic pressures, and time, i.e. aging of mycelium (Cotty, 1987; Rotem and Bashi, 1969; Smith and Berry, 1974; Turian, 1974). Some attempt has been made to find a general hypothesis to tie these disparate factors together, for example, through the inhibition of vegetative growth (Rotem and Bashi, 1969; Smith, 1978). Thus a number of environmental factors may limit vegetative growth and induce sporulation through their influence on essential growth-limiting reactions. Sporulation was suggested to be a morphogenetic expression of the Pasteur effect by Smith and Berry (1974), Smith (1978), among others, its occurrence regulated by the balance between oxidative and glycolytic pathways, most conspicuously by nutrient depletion of the substrate. Cole (1986) more cautiously states that sporulation and vegetative growth may be antagonistic, but not necessarily incompatible, using alternate metabolic pathways, but competing for the same metabolic intermediates he thought.

Responding to this type of vegetative growth versus sporulation mechanism would cause fungi to maximise vegetative growth, and therefore substrate depletion, before diversion of resources into reproduction. The onset of unfavourable conditions, such as nutrient depletion, would stimulate sporulation when it is most required for the continuation of the fungus. This represents the best strategy for fungi competing with other fungi within a substrate, for example soil fungi, but would not necessarily be so

for other fungal life styles. A few workers have found different relationships between growth rate and sporulation, some plant pathogenic fungi in particular showing abundant sporulation while vegetative growth was vigorous (e.g. Hawker, 1966). Since many plant pathogens compete only against themselves, the best strategy might therefore be to sporulate and spread to other substrates as quickly as possible, well before the substrate was depleted.

Aquatic hyphomycetes, because of the nature of their habitat, might be expected to respond to different stimuli from terrestrial fungi. If most leaves are lost downstream before decay is complete, inhibition of sporulation until the substrate is depleted would result in loss of the fungi from the stream. Thus some sporulation under normal growth conditions would be advantageous, although limited presumably by a requirement for maximal growth rates, as the fungi are also competing with other fungi and microorganisms for a limited substrate. In situ measurements of spore production from leaves in streams clearly showed that some sporulation was occurring during leaf decay, and in the nutrient enrichment experiment it appeared that vegetative growth and sporulation could occur at the same time on leaves transferred from high (aquatic hyphomycetes inhibited) to low dissolved nutrient conditions (5.3.2).

In the relationship between sporulation, nutrient levels, and vegetative growth rate, however, the pure cultures studied here were closely similar to common terrestrial fungi previously studied, for example, by Martinelli (1976) and Righelato et al (1968). Martinelli found *Aspergillus nidulans* could be induced to sporulate by either C or N starvation, but that maximum spore production occurred under conditions of C source exhaustion combined with excess N. The same pattern appeared for *L. cymbiformis* in the liquid culture experiment (4.1.2), although N was not distinguished

from the other mineral nutrients present, only from C.

Righelato et al (1968) found a relationship between C concentration and amount of sporulation for *Penicillium chrysogenum* similar to that seen in the malt concentration experiment (4.1.2). In both sporulation was induced by lowering the glucose concentration below a certain point. The higher the initial concentration had been (that is, the greater the difference between growth and sporulation conditions), the more rapidly spores were produced. The higher the glucose concentration during spore induction (to a certain point, above which sporulation declined rapidly) the greater the number of spores were produced.

The effect of different concentrations of more complex carbohydrate sources in submerged culture conditions was not tested methodically, although it might be assumed that the leaf agar discs, once submerged, would represent such a system (4.2.3). In this case abundant sporulation occurred from both 10 g\l leaf agar and 1 g\l leaf agar discs. Thus high levels of complex carbohydrates, unlike simple sugars, did not inhibit sporulation. Presumably rates of breakdown of complex carbohydrates are insufficient to lead to the build-up of the levels of whatever crucial metabolic intermediates which would favour the glycolytic over the oxidative metabolic pathways, in the imagery of Smith and Berry (1974). This would explain how vegetative growth and sporulation can occur at the same time in leaves in streams. However not all aquatic hyphomycete species can utilise all the carbohydrate sources present in leaves (Chamier, 1985), and in particular there is no information available for *L. cymbiformis*, *T. chaetocladium*, and *L. ovalis* sp. ined., apart from the fact that they grew abundantly on pectin, and to a lesser extent, cellulose agars. Thus levels of utilisable carbohydrates,

as far as these particular species were concerned, in the leaf agar were not known and are only presumed to have been high.

However, in conclusion, the Pasteur-effect model of spore induction does provide a mechanism whereby such disparate factors as, perhaps, nutrient depletion and excess oxygen, could stimulate sporulation of aquatic hyphomycetes in culture and in leaves collected from streams.

Spore production from agar pieces incubated in deionised water probably declined for the obvious reason that the energy resources of the mycelium had been exhausted in the absence of a food source.

Sporulation from leaves collected from streams would not have declined due to nutrient limitation, the food source remaining largely unaffected by incubation. Nilsson (1964) suggested that overgrowth by bacteria and phycomycetous fungi suppressed aquatic hyphomycete sporulation, and that sporulation could be maintained by changing the water of incubation regularly. In the present work changing the water of incubation daily had no effect on the decline in sporulation (4.3.5). It seems more likely therefore either that spore production simply returned to the previous level as the original stimulus to increased sporulation ceased to have an effect; or alternatively, that spore production declined as the internal resources of the mycelium available for sporulation were used up.

7.2.2 The sporulation to mycelium conversion: validity and limitations.

The present work (7.2.1 above) has been unable to demonstrate that aquatic hyphomycetes on agar and in leaves were responding to the same stimulus when sporulating. Indeed it seems more likely that different stimuli were involved. Much further work would be

necessary to prove that the sporulation response was the same independent of the inducing factor, although the close correspondence between the decline in stainable mycelium following sporulation and the mycelium/spore figures obtained from the same species on malt agar and on leaf agar (nutrient limitation being important on the former but presumably not on the later) suggests that this is the case. For instance, if it could be shown that the energy and/or resources for sporulation were derived from a conversion of those stored in vegetative biomass into spores (perhaps until a minimum level of remaining biomass was reached), then the number of spores which could be produced would depend on the vegetative resources, or biomass, present initially. Thus the maximum total number of spores inducible (possibly "switched on" by a number of inducing factors) from a given vegetative biomass might be expected to be constant within a species, giving a theoretical basis for using sporulation as a measure of biomass. Providing the inducing factors were all immediately effective, the same time scale of spore production would be followed, so that the maximum rate of production (measured in the present work) would be proportional to the total spore production.

In the present work it was shown that the amount of "live" (stainable) mycelium present prior to sporulation had decreased markedly by the time sporulation was largely over (4.1.2). The change in the amount of stainable mycelium could relate to the increased age of the mycelium following one or two weeks sporulation, although mycelium taken from areas of *A. pulchella* colonies already several weeks old showed the same percentage of stained hyphae as from younger areas. This species tends to be very slow growing, however, so that a significant proportion of the mycelium in the so-called "young" discs might perhaps have reached

the same physiological state as that in the "old" discs. Thus an age effect as the cause of the reduction in stainable mycelium after sporulation cannot be entirely ruled out.

One to two weeks in deionised water may have killed much of the mycelium or induced a strict recycling of cell contents, thus a starvation effect might also explain the reduction in stainable mycelium. Righelato et al (1968) found a maintenance level of glucose was required to prevent autolysis after growth was stopped by removal of all glucose. However, in the case of the leaf agar, the C source was presumably not soluble, so would not have been diluted in the incubation water. Any starvation effect should at least have been reduced, but in fact stainable mycelium was reduced to the same extent on leaf agar as on malt agar. Thus the reduction in stainable mycelium may relate to sporulation itself. It is suggested that even when some insoluble food sources are present, the energy and resources for sporulation are probably derived largely from the vegetative mycelium, at least under the standard conditions used in the present work. This provides a possible theoretical basis for a quantitative relationship between vegetative biomass and sporulation, but requires further work.

In the absence of a firm theoretical basis, the application of conversion factors developed from pure cultures to sporulation from naturally-colonised leaves from streams is uncertain. However, the most important factors which could have differed between leaves and pure cultures on agar have been tested and found to have no significant effect on the mycelium:spore ratio (4.2.2 -4.2.4). These were: C source and concentration (including leaf agar) during growth and sporulation, the concentration of other nutrients during growth, and the presence of natural competitors. The effect of pH was not tested, although because of the large incubation

volume:sample size ratio the pH under which sporulation was measured in the present work was probably not greatly-different whether agar discs or leaf pieces were present, in both cases being close to that of the deionised water used for incubation.

In addition, the use of sporulation as a measure of mycelial content was able to be backed up by the use of respiration measurements when pure cultures were grown on sterile leaves (4.2.8).

Factors varying between leaf samples or between streams, that is preconditioning factors, could also have affected the relationship between vegetative mycelium and sporulation, at least limiting the potential use of sporulation as a measure of mycelial content. The effect of oxygen concentration was tested for (4.2.5), together with a rough indication of the potential for leaves and streams of differing nutritional contents to affect sporulation (4.2.2 and 4.2.3). No significant effect was found from any of these. A few factors were not tested, notably the effect of the phenol content of leaves, or of individual nutrients such Ca in stream water, or of stream pH. However, the constancy of the relationship between biomass and sporulation with respect to all the tested factors suggests that it would also be unaffected by these factors.

The final result of the measurements and testing was a list of conversion factors to turn spore counts into "live" mycelial length estimates (mm trypan blue stainable mycelium). These are listed here as they were used in the leaf bag experiment (Table 7.1). The experimentally-determined figures for a range of species are given first, followed by other species (which occurred frequently in the field but which were not obtained in culture) with similar sized spores produced in a similar way, or closely related, and therefore likely to have a similar mycelium/spore relationship.

Table 7.1: "Live" (stainable) mycelium/spore conversion factors (mm/spore) calculated for 12 species in the present work. Species assumed, in the leaf bag experiment, to have similar conversion factors are given beside the appropriate species.

Species	Mycelium/spore (mm/spore)
A. pulchella	70 (Alatospora spp, C. longibrachiata, Unidentified s/r 1 (3).)
A. filiformis	4.4
A. longissima	70
A. moniliformis	0.98
A. tetracladia	3.4 (F. eccentrica, L. aquatica, T. marchalianum)
C. aquatica	7.0
Filosporella sp.	1.8
F. penicillioides	0.15 (Unidentified s/r 1(2).)
L. cymbiformis	1.4 (Lunulospora spp.)
L. ovalis sp. ined.	1.4
T. chaetocladium	14 (T. elegans)
V. elodeae	3.1

These were the minimum mycelium/spore figures obtained, except where more than one or two measurements were made, in which case an approximation to the mode was used. In species such as *L. cymbiformis* and *T. chaetocladium*, which were used in a large number of experiments, the frequency distribution of mycelium/spore values was clearly skewed, possibly as a result of the tendency to underestimate maximum spore production through too infrequent sampling being greater than the tendency to make any other experimental errors.

These mycelium/spore conversion factors (Table 7.1) were calculated from the total (stained and unstained) mycelium/spore figures obtained for each species adjusted to the average % mycelium stainable for several species (4.2.1 and 4.1.2) before sporulation. Thus differences between species in the fraction of the total mycelium stainable with trypan blue in water were not accounted for in the present work, the average percent stainable mycelium being unmeasured for most species. Of those that were measured, some species, such as *T. chaetocladium* and *A. pulchella*, showed only about 50 % of the total mycelium stained, compared to about 80 % in *L. cymbiformis* and *C. aquatica*. Thus the percentage of total mycelium stainable may be a species specific characteristic. However, relatively few measurements of each species were made, and *L. ovalis* sp. ined. showed considerable variation in percent total mycelium stainable (before sporulation) between the two experiments in which it was measured (4.1.2). Also, because growth rates, and therefore the width of the growing margin, varied between species, part of the variation in percent total mycelium stainable before sporulation could be attributable to the disparate average age of mycelium sampled in a 0.5 cm disc from the edge of a colony.

The relationship between genetic relatedness was less evident than that between spore size and mycelium:spore ratio. This was clear following calculation of the amount of mycelium per volume of spores (mm per mm^3 of spore), and where hyphal lengths were available, the mycelial volume per volume of spores (mm^3 per mm^3 of spore), as shown in Table 7.2.

Table 7.2: Lengths and volumes of stainable mycelium per mm^3 of spores produced from the mycelium over one day at maximum sporulation rates (x 10^6 mm/mm^3 and x 10^6 mm^3/mm^3).

	Length	Volume
A. pulchella	700.0	549.8
A. filiformis	6.2	11.0
A. longissima	21.2	337.2
A. moniliformis	4.9	8.7
A. tetracladia	6.8	12.0
C. aquatica	2.3	-
L. ovalis	3.5	11.0
Filosporella sp.	3.6	14.3
F. penicillioides	1.5	2.7
L. cymbiformis	2.8	8.8
T. chaetocladium	7.0	22.0
V. elodeae	1.8	-

There was a remarkable similarity in all but a few species in the amount of mycelium required to give rise to a set volume of spores (about 10 x 10^6 mm^3 mycelium per mm^3 of spore). The species that differed were *A. pulchella* and *A. longissima* both apparently being much less sporuliferous. The figure for the amount of stainable mycelium per spore could be reduced somewhat by assuming that the amount of stainable mycelium in these species was less than the average (for other species), perhaps half as little again (4.1.3). However, this could not explain all the difference. Thus the high mycelial volume:spore volume ratio of these species may result from a real difference in reproductive strategy, although a very significant error in the measurement of sporulation in culture

cannot be entirely discounted. The mycelium/spore relationship was measured only once for both these species, although separate measurements were made for mycelium-from old and young parts of the colonies.

The volume of mycelium apparently giving rise to 1 mm^3 of spore in *F. penicillioides* is somewhat lower than that for other species, although not very greatly. Errors could have entered the calculations particularly in the estimates of hyphal radii and spore volumes. The radii of hyphae vary considerably, depending on a number of factors, including whether submerged (in agar) or aerial. The radius used to calculate mycelial volume was obtained from buried hyphae, while *F. penicillioides* in particular, had abundant, very broad, aerial hyphae. The spore volume measurement was obtained from a different isolate, and not from the actual spores produced during measurement of spore production. Spore sizes vary greatly even within an isolate. The spore volumes in all the above species were only estimated roughly for the mid point in the range of spore sizes measured during taxonomic studies, not for a true average of the spores produced in the sporulation measure.

The fact that, despite these potential sources of error, most species show a similar relationship between the volume of mycelium and the volume of spores produced from it has important implications for the use of spore production as a measure of the mycelial content of leaves. It suggests that the relationship between vegetative biomass and spore production is a very basic and reliable physiological property.

However, at this stage, because *A. pulchella* and *A. longissima* had such different mycelial volume:spore volume ratios, the technique (using spore production as a measure of mycelial content)

must be limited for future work by the requirement for mycelium/spore values to be calculated for each significant species present in a litter sample. These should also be tested before use with specific treatments that might affect the relationship between mycelium and sporulation, for example, in experiments to measure the effect of toxins on aquatic hyphomycetes (Bärlocher and Premdas, 1988).

The technique is also limited to some extent as "biomass" is calibrated in mycelial lengths. Several workers have used mycelial lengths to obtain biomass in grams (Kjøller and Struwe, 1982) or to obtain fungal C (Findley et al, 1986). Both require the use of several further conversion factors (average cross sectional area of hyphae, hyphal density, and moisture content), all of which will also vary between species and with growth conditions. Because of the amount of work involved in testing and measuring these no attempt was made to convert mycelial lengths in the leaf bag experiment to either of these forms.

Total mycelium can be divided into stained and unstained, or live and dead hyphae. The two methods of division do not coincide exactly, depending on the stain used (Parkinson, 1973; Cohen, 1984; Schnürer et al, 1985). Trypan blue in water, the stain used in the present work to measure stained mycelium/spore figures, did not appear to stain fungal cell walls, thus distinguishing between hyphae with and without cell contents. All hyphae with contents were not necessarily alive, however.

The most useful measure of fungal biomass in a study will depend on the purpose of the study. For example, if considering fungal production per year on a food source, total mycelium may be the most meaningful measurement. In a study of the importance of fungi during leaf decay, such as the leaf bag experiment, the most

relevant measure is amount of live fungi, capable of actively degrading leaf material present at any one time. Although the mycelium/spore figures calculated in most experiments in section 4.2 were for "total mycelium/spore" they will only give accurate total mycelium figures from sporulation measurements where the ratio of total to live hyphae is the same as in the agar cultures, as sporulation can only derive from live mycelium. Thus only stainable mycelium could probably be reliably estimated from sporulation in the leaf bag experiment, and even this required the assumption that the ratio between stainable and live mycelium was not significantly different from that of the same fungi in pure culture.

7.2.3 Comparison with other methods.

The range of alternative methods for determining aquatic hyphomycete biomass (either in total or of individual species) is not great. Some workers have used direct microscopic measurement of hyphal lengths in leaves to determine total fungal biomass, for example, Bärlocher and Kendrick (1974). Alternatively, selective inhibition of respiration followed by conversion of respiration data into biomass could give a figure for total fungal biomass. Frequency of isolation data could perhaps then be used to estimate the biomass of the aquatic hyphomycetes or of individual species.

In the present work leaf discs were cleared and stained in an attempt to measure hyphal lengths, without success. Mycelium was not visible inside intact leaves. Fungal structures were difficult to distinguish from algal, leaf, and various amorphous structures, even without maceration, and chitin stains (McLean and Cook, 1958) did not give much improvement. It was concluded that mycelial measurement techniques could not be applied to decaying leaves with

any accuracy.

Obtaining frequency of isolation data is very time-consuming, and has several inherent weaknesses. In order for it to measure the relative proportions of aquatic hyphomycetes and other fungi, all fungi present and active must be detected equally. However, both the plating technique and whatever medium is used are selective, favouring fungi that grow fast and compete well, and which sporulate on artificial media to allow identification. Dick (1976) pointed out the effectiveness in obtaining various phycomycetous fungi by baiting and trapping procedures compared with standard plating methods. The Saprolegniaceae and Leptomitaceae, for example, compete very poorly with other fungi, particularly on dilute isolation media. Thus the rarity or absence of these groups from the present plating experiment does not mean they were not present, or even important in decaying leaves, and contributing to fungal respiration.

The plating technique does not distinguish between fungi present as active mycelium and those present in dormant forms. For example, Park (1974) demonstrated that propagules of common soil fungi were washed into streams and became impacted on the surfaces of objects. In addition, fungi already growing in leaves when they enter a stream could survive for some time, although inactive and unimportant to leaf decomposition (Bärlocher and Kendrick, 1981).

Thus frequency of isolation data will not necessarily give an accurate subdivision of mycelial lengths or respiration into that attributable to different fungal groups.

However, bearing in mind the above limitations, the figures for frequency of detection by plating in the present work (section 6.2) were useful as an indication that some fungi other than aquatic hyphomycetes were present in abundance, thus confirming the combined

results of the S.I.R. experiments and aquatic hyphomycete sporulation from randomly collected leaves as to the relative importance of aquatic hyphomycetes (6.1.3). The plating data were also used to test the mycelium/spore values obtained in the present work, by comparing the percent composition obtained from the figures for frequency of detection of different aquatic hyphomycete species with the percent composition obtained from the sporulation results and from the figures for mycelial content (6.2.2). If the mycelial lengths obtained from the sporulation figures were indeed a better measure of the actual mycelial content than the sporulation figures themselves, then the relative importance of different species in the plating experiment might be expected to be closer to that of the mycelial lengths than the spore counts.

This will only be the case, however, if all three methods were equally efficient at sampling the species. Some species were poorly detected by the plating technique as practised in the present work, most notably small spored, sparsely sporulating species such as *Alatospora* spp. This is a pity, as the mycelium/spore value for *A. pulchella* is one which particularly requires checking, apparently being so much larger than that for most other species. The percent of both the total spore production and biomass belonging to this species were greater than the percentage of the plating flora. In contrast, species which sporulated poorly under water (some Unidentified sigmoid/rod types) appeared to be more abundant in the plating experiment than in the sporulation measure.

Of the remaining species, the percent of the plating flora which was comprised of *Lunulospora* spp., *L. ovalis* sp. ined., Unidentified s/r type 1(2), *T. chaetocladium*, and *C. aquatica* showed a closer

correlation with percent of total mycelial content than percent of total sporulation. Thus the plating data for the dominant species in the Hakarimata stream and Reid's Ck support the biomass results obtained from sporulation.

In contrast *A. tetracladia* (together with *F. eccentrica* and *L. aquatica*, species which were not clearly distinguished between), *A. filiformis*, *H. lugdunensis*, *T. marchalianum*, and *V. elodeae* comprised much higher percentages of the plating flora than of the total spore production and mycelial content (or sporulation alone where only this was available: *H. lugdunensis*). All the aquatic hyphomycete species tested in the present work sporulated at least as well, and usually better, when submerged, and these included all the above species except *H. lugdunensis* (which was not tested), indicating that the difference in importance between plating- and sporulation-measured floras was probably not caused by a difference in sporulation physiology. Each of these species was recorded by the sporulation technique at percentages as high as those found in the plating experiment at some stage in the leaf bag experiment or stream survey. Thus such figures were not impossible to obtain with the sporulation technique, further suggesting that the difference in frequency was not due to any inadequacy in the sporulation method.

Another possibility is that the mycelium/spore conversion factors used for the species were too small, and that with the use of the correct one the percent of total mycelial content would have matched that of the plating flora. In the case of *H. lugdunensis* no mycelium/spore figure was available, but in view of the very abundant sporulation seen in the isolation plates, and the moderate size of the spore, it seems unlikely that the mycelium/spore value

for this species would be sufficiently high. The mycelium/spore conversion factor for *A. tetracladia* would have to have been 10 times that measured to give mycelial lengths which could compare to the frequencies seen in the plating experiment. This seems unlikely also, as this would require fewer spores to be produced per unit of mycelium than *T. chaetocladium* for example, which has larger spores produced on less branched conidiophores. The mycelium/spore figure used for *A. filiformis* could perhaps have been too low, but from Table 7.2 it would appear that it does not differ from most of the other species in the relationship between mycelial volume and spore volume.

Thus the cause of the difference between plating and mycelial content and/or sporulation percentage compositions in some species could be explained in some cases by differences in the efficiency of either the sporulation or plating method rather than inaccuracies in the mycelium/spore values used to calculate the mycelial content, but was not immediately obvious in others.

In conclusion, the sporulation technique, with appropriate mycelium/spore conversion factors, appears to be a useful technique for measuring the aquatic hyphomycete mycelial content in leaves. It has all the high variability and necessary assumptions of any indirect measure, but these are outweighed by the convenience of the method, particularly its relative rapidity compared with alternative methods. It may be most useful for those whose primary interest is in individual aquatic hyphomycetes species, but is possibly also useful in studies of energy dynamics and community interactions where a mycelial measure for the whole fungal group is of more interest. Both applications were utilised in the leaf bag experiment, in the present work.

CHAPTER EIGHT: DISCUSSION OF LEAF DECAY PROCESSES AND AQUATIC HYPHOMYCETE ECOLOGY.

The major aims of the present work were to study leaf decay in two New Zealand streams, and associated aspects of aquatic hyphomycete ecology, namely the occurrence during decay of aquatic hyphomycetes on different leaf types, and in different seasons and streams. These aims are addressed here. The sporulation technique developed to measure aquatic hyphomycete mycelial content for this work has been discussed in the previous chapter, as have the inhibition of respiration and particle plating methods which were used to measure the relative importance of aquatic hyphomycetes compared to other microbial groups. Most of the data discussed in the present chapter are reported in Chapter 5.

8.1 THE LEAF DECAY PROCESS IN NEW ZEALAND STREAMS.

8.1.1 Description of Rates of Weight Loss.

A number of methods have been used in the past to describe the pattern of weight loss in decaying leaves. The simplest system (Petersen and Cummins, 1974) divided leaves into "fast", "medium", and "slow" decaying species on the basis of the length of time to 50, 80, and 90 % weight loss. More complex systems have described weight loss by linear and exponential models, and the rate coefficient used to compare leaf types. Mutch and Steadman (1983), for example, found a linear function gave the best fit for willow leaf decay data between day 2 and 80 days, that is, weight loss per

unit of time remained constant throughout almost all of decay. McCammon (1980) tested a linear, negative exponential model (assumes a constant fraction of the weight remaining lost per unit of time), and a positive exponential model (assumes an increasing fractional loss of weight) on decay of mountain beech (*Nothofagus solandri* var. *cliffortioides*), and found the positive exponential model fitted best. Hanson et al (1984) produced a two variable negative exponential model which incorporated temperature as the second independent variable, and found this an improvement over a single variable model.

In the present work it would appear that the weight loss relationship is more complex than in any of the models used above. A similar biphasic curve in decay rate occurred in both runs (Figs 5.2 and 5.3), although the temperature change during decay (due to season) was reversed, indicating that temperature was not the causal factor. It will be shown later that these variations in decay rate probably represented real, biologically significant stages in decay, resulting from the relative importance of several causes of weight loss.

The most meaningful method of comparing decay rates in different leaf types in the present work would therefore be either by graphing them side by side, or by comparing time taken for set amounts of weight loss to occur, as did Petersen and Cummins (1974). According to their categories:

	50%	80%	90% weight loss
"fast" =	<46 days	<107 days	<230 days
"medium" =	46 -138 days	107 -322 days	230 -461 days
"slow" =	>138 days	>322 days	>461 days

The corresponding figures obtained in the leaf bag experiment are

given below in Table 8.1.

Table 8.1: Time taken (days) for leaves placed in the Hakarimata stream and Reid's Ck in summer (first run) and winter (second run) to reach 50, 80, and 90 % weight loss. The leaf types used were kamahi, silver beech, hangehange, kanono, and kauri, the later two as young, newly formed leaves, and as older leaves from previous season(s).

	HAKARIMATA			REID'S CK		
	50 %	80 %	90 %	50 %	80 %	90 %
kamahi 1st run	62	139	162	82	136	160
kamahi 2nd run	104	208	270	190	230	276
beech 1st run	82	130	139	173	240	289
beech 2nd run	135	181	214	214	272	298
hangehange	28	52	62			
kanono (young and old)	52	107	121			
kauri (young and old)	361	503	561			

Thus only hangehange belonged to the "fast" decaying category. Kamahi, beech and kanono fitted into the "medium" category, except for second run kamahi and both beech runs in Reid's Ck, where they were "slow" species. Kauri was a "slow" species, as, evidently, were rimu, nikau, ponga, and tawa (Table 5.1).

In order to compare leaf types on the basis of time taken to reach a certain percent weight loss, fixed percents need to be chosen. The best choice of percent weight loss(es) to use would be at the end of each biologically significant stage of decay, for example immediately after each of the peaks in decay rate (Figs 5.2, 5.3). These would give most information regarding decay rate at

comparable stages, and also the relative importance of each stage to a particular leaf type. However, the percent weight loss to which each of these stages of decay correspond, as indicated by changes in decay rate or aquatic hyphomycete species composition, varied somewhat between leaf types and seasons. For example, 50 % weight loss occurred several months before the second peak in decay rate in first run kamahi, but coincided with it in second run kamahi and in beech in both runs (Figs 5.2 and 5.3 compared with Fig. 5.1). The most extreme example was ponga, which at 25 % weight loss had a fungal flora most like that of kamahi at 93 % weight loss. Thus the information obtainable from the 50, 80, and 90 % weight loss figures is the same as that from decay rate coefficients. Both compare only the relative average decay rate between different leaf types. This being the case, only one figure is necessary, 50 % weight loss being the most useful. It is the fastest to obtain in leaf bag experiments, and probably the most ecologically important, being the most frequently reached among naturally occurring leaves before they are carried away downstream. It is also the least variable, as variation between consecutive weight loss results appeared to increase later in decay.

The differences in decay category (Table 8.1) seen for kamahi and beech between streams and season indicate that temperature needs to be included in the definition of "fast", "medium", and "slow". Thus decay of hangehange was "fast" at an average temperature of 13.5 C. Kanono, kamahi, and beech in Hakarimata were "medium" decay species at average temperatures of 12 and 13 C (first and second runs), while kauri, rimu, nikau, ponga, and tawa were slow species at an average yearly temperature of 11 C. Kamahi in Reid's Ck was a "medium" decay species at average temperatures of about 10 C, and a "slow" species at about 7 C. Beech in Reid's Ck was a slow species at both temperatures.

8.1.2 Factors Affecting Decay Rate.

The time taken by the leaves in the leaf bag experiment to decay varied between leaf types, streams, and seasons. The differences in decay time observed between seasons and between streams (higher in summer and in Hakarimata) may relate simply to temperature, but other factors could be involved in the differences between streams in particular. The concentration of nutrients in the stream water has the potential to influence the decay rate of organic materials (e.g. Melillo et al, 1983b). Nutrient levels in Hakarimata, as a lowland stream draining a larger standing crop of forest, may be assumed to be higher than those in Reid's Ck, so contributing to the higher rates of weight loss in the former. The sites chosen in each stream were both pool sites, but the total amount of flow through each may well have differed, affecting the amount of physical wearing, and therefore the decay rate. Benfield and Webster (1985) found that the difference in decay rate between first and second order streams correlated with shredder abundance. The composition of the invertebrate fauna may also have differed between seasons or streams, particularly the proportion capable of active consumption of the leaves in their particular decay phase, for example, the small worms and insect larvae that burrowed in the first run kamahi leaves.

Species specific rates of weight loss have been found frequently in previous work. They appear to relate to the rate at which a leaf type becomes colonised by microorganisms and then palatable to invertebrates. The rate at which this occurs depends on the leaf chemistry and structure. Bärlocher et al (1979) found needles of *Pinus leucodermis* Ant. were only slowly colonised until methanol soluble inhibitors present in the needles were lost. The loss of these (through leaching) was slowed by well developed lipophilic outer layers: the cuticle and associated waxes. Suberkropp et al

(1976) found levels of inhibitory phenolic compounds to be more important to-weight loss of hickory compared with oak leaves than concentrations of cellulose, hemicellulose and lignin. No information on phenolics or other inhibitory compounds was available on the leaf types used in the leaf bag experiment, but kauri, being a conifer, probably has relatively high levels, and certainly the leaves are thick, with a well developed, rigid cuticle and epidermis which would slow the loss of such compounds.

Nutrient content can affect the rate of decay of organic materials. Either or both of N content and lignin content have been linked to rates of decay of different leaf types (Marinucci et al, 1983; Rogers and Breen, 1982; Melillo et al, 1983a; Melillo et al, 1983b; Bird and Kaushik, 1987). The lignin content of the present leaf types was not known, but some data on the N content of the leaf types used in the leaf bag experiment were available from the literature (Table 8.2).

Table 8.2. Energy content (kJ/g) and N content (%) of some of the leaf types used in the leaf bag experiment. References: Bagnall (1972), Fitzgerald (1976), Heine (1973), Madgewick et al (1982), and Peterson (1961). No information was available on kanono.

	Energy Content	N
hangehange	17.1	3.36
kamahi	20.4	1.31
beech	20.4	1.9 -2.6
young kauri	21.8	0.59 -0.96
old kauri	22.2	0.36 -0.87

Thus decay time followed an inverse pattern among leaf types to

N content. This suggests that N content was important in determining the time taken to decay of a leaf type in the present work. However, the energy content of the leaf types (Table 8.2) follows an exactly inverse sequence, as, probably, do other factors such as cuticle thickness and amount of lignification. This indicates that some factor which energy content measures, e.g. lipid content, could also be, at the very least, an additional determining factor.

8.1.3 Variation in Decay Rate During Decay.

The absence of replication of weight loss measurements in the present work contributed to the conspicuous variation in % weight loss between consecutive samples shown in Fig. 5.1. Obvious physical differences in stage of decay were seen several times between leaves in replicate bags, and between individual leaves in the same bag. These probably relate to differences in microenvironment such as exposure to water flow (physical wearing), large invertebrates being trapped in the mesh bags, and to differences in age, thickness etc of the initial leaves. The same factors were probably responsible for some variation in the abundance of aquatic hyphomycetes e.g. the May sample of first run kamahi in Hakarimata showed very low spore production compared with the April and June samples (Table 5.6). Chamier and Dixon (1982) and Chamier et al (1984) also noted variation in the rate of leaf decay which resulted in occasional samples being out of phase in terms of number of spores/g. A smoothing function could have been used on the weight loss data, but was rejected on the grounds that it would also smooth out differences in decay rate which were obviously not random, since they were repeated in different runs and

streams.

Decay rate (Figs 5.2, 5.3) varied with time, appearing as four stages: an initially high rate, followed by a period of lower rates, then a second peak, and a final decline to low rates. These stages can be related to changes in the relative importance of different causes of weight loss:

1) The initially high rate of weight loss probably resulted from leaching and loose particles washing off from the leaves. Leaves became waterlogged, and in some cases slimy to touch. Respiration, aquatic hyphomycete mycelial content and invertebrate biomass were all initially low, suggesting the potential for active consumption of leaf material was not great. According to Kaushik and Hynes (1971) most of the weight loss due to leaching of already dead leaves occurs in the first day or few days and is relatively unaffected by water temperature. Thus it seems likely that the inital peak in decay rate, recorded in the first month, may include some active utilization of perhaps the simpler leaf components by the microorganisms or invertebrates beginning to colonise the leaves. The rate at which colonisation occurs varies between seasons and streams, and this may explain the fact that the initial peak in decay rate was highest in first run leaves in Hakarimata, when initial respiration rates were highest.

When the water temperature was low there was a noticeable lag prior to this peak in decay rate, probably because leaf death and infiltration took longer. Thus spreading out the initial leaching weight loss over the first two months, occasionally more, may also account for some of the difference in weight lost in the first month in Hakarimata compared with Reid's Ck, or the first compared with the second run.

2)The relatively low decay rates which followed the first peak in decay rate corresponded to a period of colonization and conditioning by microorganisms. Leaves became softened, or dark and rigid, according to leaf type. Respiration rates increased through much or all of this period (Figs 5.4 -5.11). Aquatic hyphomycete spore production (and mycelial content) and invertebrate biomass increased also (Figs 5.4 -5.11 and Tables 5.2, 5.3).

The weight loss calculated as resulting from microbial respiration is given in 8.1.4. From these calculations it appeared that microbial respiration was responsible for a greater proportion of total weight loss during the first half of decay (corresponding to the postulated period of microbial colonisation and conditioning) than over complete decay. This supports the concept of an early decay period dominated by microorganisms.

3)The second peak in decay rate corresponded to greatly increased invertebrate consumption and fragmentation of the then soft or brittle, optimally conditioned leaves. Both consumption (chewing the whole leaf or burrowing within it) and physical wearing and fragmentation were visible in the leaves. Maximum decay rate generally coincided with the peak in invertebrate biomass, which generally occurred slightly after the peak in spore production and mycelial content (Figs 5.2 and 5.3 compared with Tables 5.6 and 5.2, 5.3). The connection between leaf decay and invertebrate biomass was most obvious in the case of kauri, where invertebrate biomass increased after a certain amount of weight loss (amount of conditioning) rather than after the same length of time immersed as the other leaf types. The increase also coincided with the first visible loss of leaf area.

4)Following the second peak in decay rate there was a gradual decline as progressively less palatable, more resistant leaf parts remained. Frequently leaf veins and petioles were observed to persist after most or all of the interveinal tissue was lost. During this period respiration, where measured, was low, and spore production and invertebrate biomass declined steadily (Tables 5.4, 5.5, 5.6 and 5.2, 5.3).

8.1.4 Contribution of Microorganisms to Weight Loss.

Petersen and Cummins (1974) measured or calculated the weight loss due to leaching, physical wearing, microbial action, and invertebrate consumption for hickory, a species with a medium decay rate:

leaching	15 %
conditioning (30 days)	7 %
subsequent microbial utilization	15 %
invertebrate utilization	21 %
lost into particulate detritus pool	41 %

Of these, weight loss due to microbial metabolism was of primary interest in the present work. Petersen and Cummins (1974) calculated this weight loss from leaf respiration measurements, using the method outlined by Southwood (1966).

The same method was applied here, using an energy equivalent for oxygen of 20.1 J/ml (RQ = 0.80), and energy contents of 20.4 kJ/g for kamahi and beech (Fitzgerald, 1976 and Bagnall, 1972). The weight loss attributable to respiration by microorganisms over the whole of leaf decay and over the first half of leaf decay are shown in Table 8.3.

Table 8.3: Weight loss from kamahi and beech leaves in the Hakarimata stream and Reid's Ck due to microbial respiration during the first half of decay (initial) and during the whole of decay, expressed as a fraction (%) of the actual, measured, total weight loss during that period.

	HAKARIMATA		REID'S CK	
	Initial	Total	Initial	Total
first run kamahi	100 %	61 %	66 %	48 %
second run kamahi	47 %	35 %	49 %	30 %
first run beech	71 %	41 %	61 %	34 %
second run beech	62 %	40 %	?	46 %

All the figures are likely to be slight overestimates as oven dry weights were used rather than ash free dry weights. Winterbourn (1982) found the difference between the dry weight and ash free dry weight of beech litter to be only 7 %.

The choice of energy equivalent for oxygen used by Petersen and Cummins (1974) was not explained, but is possibly slightly low, e.g. Elwood et al (1981) used 20.6 J/ml (RQ = 0.9). The same figure as that used by Petersen and Cummins was used in the present work, to allow a comparison with their results. However this may have resulted in a slight underestimate of the microbial contribution to weight loss.

There is likely to be a high variability built into these figures due to several factors: there was no replication of the respiration measurements, and on occasion the respiration of microinvertebrates within the leaf sample may have been included, as the leaves were only gently rinsed prior to the respiration measurements. Although respiration was measured at stream

temperatures, in stream water, and without cutting the leaves up, factors during collection or transportation may have altered the field microbial populations and their respiration rate prior to the measurement of respiration.

The most useful figure from this section of work is therefore the average fraction of the whole of decay attributable to respiration of microorganisms, i.e. 42 %. This is higher than that found by Petersen and Cummins (1974): 15 + 7 = 22 %. There is some indication of higher percentages occurring in the first run (summer) leaves compared with the second run. As Petersen and Cummins carried out their work in autumn, the average figure for the second run leaves (38 %) was, as might be expected, slightly closer to theirs. Elwood et al (1981), testing the effects of low and high levels of P enrichment on oak leaf packs, calculated that 10 and 34 % of the increased weight loss was attributable to the increase in microbial respiration. The results of the present work lie close to the upper end of this range.

The figures in Table 8.3 give only part of the total weight loss due to microorganisms, and do not include the leaf material fixed in microbial biomass and consumed by invertebrates or worn away, that is, lost in any way that does not result in microbial respiration. This fraction is hard to calculate.

Kjøller and Struwe (1982) quoted an average production efficiency of 0.40 for soil fungi. The range was 0.1 -0.8, depending on the amount of maintenance energy required. In other words, when the growth rate is low the proportion of the substrate consumed which is used for maintenance (respired), rather than fixed in biomass, is high, and vice versa. Assuming that the same production efficiencies apply in aquatic litter, this implies an average total microbial utilization of 46 % (production efficiency =

0.1) to 100 % (production efficiency = 0.4 or greater) of the leaf material in the leaf bag experiment during the entire weight loss period. This range seems unrealistically high, as observation of the amount of chewed and fragmented material present during decay suggested that both invertebrate consumption and physical wearing had important roles in weight loss. Also, peak respiration rates coincided with a period of low weight losses (Table 5.4 and 5.5 compared with Figs 5.2 and 5.3). However, very low production efficiencies (and low growth rates) also seem unlikely. It is possible that a significant fraction of the substrate fixed in microbial biomass may be rapidly recycled, eventually being respired, by the same or different microorganisms. This fraction is impossible to calculate here, although Cochran et al (1988) calculated that 55 % of the C in dead microorganisms growing on low levels of glucose was recycled in new microbial material. If recycling occurred at this rate (50 %) in leaves in streams, the estimate for the average total microbial utilisation drops to 44 -67 % of the leaf material.

Not all the measured respiration in the leaf bag experiment can be attributed to decomposer organisms: Polunin (1984) noted significant microbial activity on inert surfaces, including algal growth where light was sufficient. Rounick and Winterbourn (1983b) studied the development of organic layers on stones in New Zealand streams and found respiration rates of 5 ug oxygen/cm^2/hour on substrates kept in continuous dark for two months. These layers appeared to contain bacteria and fungi. After the same length of time, respiration of beech leaves, expressed on an area basis, was 3 -10 times this figure. On this basis a significant proportion (up to a third) of the measured respiration might be attributable to purely surface -living microorganisms, unless the stream in which

Rounick and Winterbourn worked contained very high nutrient levels compared to Hakarimata and Reid's Ck, or decaying leaves do not provide a suitable site for the development of the type of organic layers found on stones, neither of which seems very likely.

Because the weight remaining in the first month or two was high the weight loss calculated from the early respiration rates has a disproportionate influence on the total figures calculated in Table 8.3. In particular, the respiration rates measured in the first month or less, largely prior to colonisation by aquatic hyphomycetes, were frequently higher than the immediately subsequent rates. This respiration may not relate to leaf decay at all.

In conclusion, the figure for microbial utilization of decaying leaf litter of kamahi/beech type during complete decay seems most likely to be not less than 29 % (44% minus the maximum contribution from surface-living microorganisms) and not more than 60 % (67 % minus the minimum contribution from surface -living microorganisms). This may vary considerably between seasons and streams.

8.1.5 Contribution of Aquatic Hyphomycetes to Weight Loss.

In the sterile leaf experiment 34 % of kamahi and 23 % of beech leaves were lost in 77 days at 13 C, following inoculation with a single aquatic hyphomycete species, indicating that the potential for rapid weight loss from the action of aquatic hyphomycetes alone is considerable.

Using the same method as in 8.1.4, the respiration rates from the sterile leaf experiment can be used to calculate the amount of weight loss due to microbial (in this case inoculated aquatic hyphomycetes) respiration. As above, the resulting figures will

tend to underestimate fungal utilisation, in that they will indicate only the leaf weight respired by the fungi, and not that fixed in the fungal biomass. However, there will also be three sources of error resulting in a tendency to overestimate the amount of weight loss due to fungal respiration: the use of oven dry weights compared with ash-free dry weight; the use of the initial dry leaf weight, when the weight of the substrate decreased during the experiment; and the use of the respiration rate obtained at the end of the experiment, when the respiration rate would have increased to this from zero. The first factor was probably unimportant, the second was minimised by using a median weight between the initial and final weight for calculation, but the third factor was more difficult to overcome. Assuming that the initial colonisation and increase in biomass would have taken at least the first two weeks, this effectively reduces the period over which respiration was occurring at anything like the rate measured at the end of the experiment. Therefore the following figures for amount of weight loss attributable to fungal respiration were calculated for a 63 day incubation period rather than the actual 77 day period:

Kamahi

L. cymbiformis = 53 % of total weight loss

T. chaetocladium = 65 % of total weight loss

Beech

L. cymbiformis = 74 % of total weight loss

T. chaetocladium = 140 % of total weight loss.

It is clear from the final figure (*T. chaetocladium* on beech) that there was still a tendency to overestimate the weight loss due to the inoculated fungi. Thus probably significantly less than 50 %

of the weight loss observed from kamahi, and somewhat more from beech, was due to active fungal utilisation. The remaining weight loss was probably due to physical wearing following softening of the leaves by fungal enzymes, e.g. pectinases (Chamier, 1985). In the present work accumulations of fine leaf particles were observed when the incubation fluid was changed, particularly in the flasks containing kamahi.

The spore numbers recorded from the sterile leaves after 2 days were very similar to, or only slightly higher than, those recorded from second-run kamahi leaves in Hakarimata, indicating that the mycelial content of the sterile and leaf bag experimental leaves was similar. However, dried leaves were used in the sterile leaf experiment. Similar dried leaves placed in the Hakarimata stream decayed more rapidly than either fresh green kamahi in the stream or the leaves in the sterile leaf experiment: 50 % compared to 30 % or less weight loss after one month at about 13 C. This suggests that aquatic hyphomycetes alone do not cause as rapid weight loss as do the varied microorganisms and invertebrates colonising leaves in streams. However, the aquatic hyphomycete mycelial content, as measured by spore production, of dried kamahi in Hakarimata was significantly greater than that measured from fresh leaves after the same time. Thus the faster decay rates may reflect, at least in part, faster colonisation. In this case the results of the sterile leaf experiment suggest that the aquatic hyphomycetes in the leaf bag experiment are responsible for most of the weight loss attributed to microorganisms generally.

This conclusion contradicts the results of the experiments involving selective inhibition of respiration, in which only about 50 % of the total microbial respiration appeared to be of fungal origin, and on average 70 % of this was attributable to aquatic

hyphomycetes. The randomly collected leaf litter used in the S.I.R. experiments may, of course, have differed somewhat from the kamahi and beech leaves in the leaf bag experiment, and was certainly composed of a greater variety of leaf types. However, the levels of aquatic hyphomycete colonisation, as measured by spore production, were closely similar to the leaf bag leaves after one to three months immersion, that is, levels of colonisation were most commonly equivalent to those recorded slightly prior to peak spore production and biomass in the leaf bag experiment.

Triska (1970) found that a bacterial fraction dominated the respiration of particular leaf types at some stages of decay, in a leaf bag experiment somewhat similar to the present one. In the present work aquatic hyphomycete mycelial content was low but respiration measurements were high on some leaf types (for example hangehange) throughout decay. Here it is reasonable to conclude that other microorganisms, such as bacteria, dominated leaf decay. However, as no separate measurements of microorganisms other than aquatic hyphomycetes were made in the present work, predictions of how the quantitative relationship between aquatic hyphomycetes and other microorganisms may have varied during decay depend largely on previous work.

It is possible that the different microbial fractions have different roles, at least in early decay, when bacterial access to the leaf interior may be limited. In microscopic examinations of cleared and stained leaves from random litter collections in the present work conidiophores were seen emerging from stomata, or apparently piercing the epidermis of intact leaves. Where leaves were partly decayed hyphae were visible ramifying through vascular bundles, or contorted to pack full individual mesophyll or epidermal cells. In contrast, bacteria were distinguished only on the

surfaces of intact leaves and cells. This may limit the role of bacteria in leaf decay, at least initially.

Previous studies showed some variation in the patterns of relative abundance of bacteria and fungi during decay. However, bacterial numbers were strongly influenced by temperature, so that decay related patterns may not be separable from temperature related patterns where leaf decay coincided with decreasing temperatures in autumn or increasing temperatures in spring, as in many studies of autumn shed leaves. Most studies found that bacteria increased in importance later in decay (Bärlocher and Kendrick, 1981; Chamier, 1987 -oak leaves), possibly coinciding with the increasing surface area of decaying leaves. Kaushik and Hynes (1968) and Kjøller et al (1985) found bacterial numbers decreased during decay. Chamier found bacterial numbers showed an inverse relationship to aquatic hyphomycete numbers on alder, being high at the beginning and end of decay only (Chamier et al, 1984; Chamier, 1987). Kaushik and Hynes (1968) quoted Witcamp to the effect that easily decomposable species show a decrease in bacterial numbers during decay, whereas initially inert species show increasing numbers. This suggests then that bacteria probably increased in number during decay on most leaf types with the exception of hangehange, kanono, and perhaps first run kamahi, particularly in Hakarimata, all of which probably had high numbers of bacteria in the early stages of decay. The interrelationship between the bacterial and fungal components of the microflora of decomposing leaves in New Zealand streams requires more work.

In summary, it seems most likely that aquatic hyphomycetes may have been responsible for at least 10 % of the total weight loss of the kamahi and beech leaves in the leaf bag experiment (minimum total microbial contribution (29 %), divided into fungal (50 %) and

bacterial on the basis of the S.I.R. work, and the fungal fraction in turn divided into aquatic hyphomycete fraction (70 %) and other fungi). They were probably responsible for considerably less of the weight loss of some of the other leaf types, which were not so well colonised by aquatic hyphomycetes, for example hangehange, ponga, and nikau.

8.1.6 Comparison with Previous Work.

The decay times of leaf types recorded in the leaf bag experiment showed at least as great a range as that found in temperate overseas studies, for example Petersen and Cummins (1974), Bird and Kaushik (1987). There has been very little work done on the decay rate of leaves of New Zealand species in streams. Davis and Winterbourn (1977) recorded 49 % weight loss in 152 days in mountain beech, a species which might be expected to show similar decay characteristics to the silver beech used in the present leaf bag experiment. Rounick and Winterbourn (1983a) found mountain beech leaves were completely decayed in 154 days in a stable South Island stream, but only 75 % weight loss occurred in the same time in an unstable (flood -prone) South Island stream. Silver beech weight loss in Hakarimata (first run) was similar to that in Rounick and Winterbourn's stable stream, but in Reid's Ck was more like that in Davis and Winterbourn's study. The differences in the present work, however, were more likely to have been caused by temperature than stream stability. McCammon (1980) recorded a considerably slower decay time for mountain beech than in any of the above examples. He measured 50 % weight loss in about 300 days, longer even than second run silver beech in Reid's Ck.

Collier and Winterbourn (1987) found rates of weight loss from kamahi in four streams varied in response to the stream pH, the time taken to reach 50 % weight loss ranging from 176 days at pH 7.5 to 321 days at pH 4.7. Thus the rate of decay in Collier and Winterbourn's circumneutral streams corresponds well to that found for second-run kamahi in Reid's Ck, which shows a similar pH and temperature profile.

These previous studies used dried leaves rather than fresh, as in the present leaf bag experiment. Rogers and Breen (1982) found that, compared to naturally senescent leaves, dried *Potamogeton* leaves showed a larger initial loss of nutrients through leaching, which in turn resulted in a changed nutrient status, reduced colonization by detritivores and microorganisms, and a slower decay rate. However, the reverse pattern appeared in kamahi placed in Hakarimata for a month at a time in the present work. Both weight loss and sporulation at 1 month in dried leaves compared favourably in most cases with that of fresh leaves after 3 months. Thus the use of dried leaves is unlikely to be the cause of the slow decay rate of McCammon's (1980) leaves. In fact the experiments with dried kamahi suggest that it is surprising that decay rates in the other previous studies corresponded as closely to those in the present work.

There has been almost as little quantification of the causative agents of decay in New Zealand, particularly microbial colonisation (respiration, mycelial content, spore production) and invertebrate colonisation, as of rates of decay. Rounick and Winterbourn (1983a) recorded peak respiration rates on mountain beech at 6 -8 weeks of 0.3 mg oxygen/g/h. Collier and Winterbourn (1987) recorded respiration rates of up to 0.07 mg oxygen/g/h from kamahi leaves. Overseas workers results ranged from 0.09 mg/g/h to 0.8 mg/g/h

(maximum rate measured) on various deciduous leaf types and conifers (Allard and Moreau, 1986, Iversen, 1973, Petersen and Cummins, 1974, and Triska et al, 1975). These maximum rates were achieved at 26 -42 days.

Peak respiration rates recorded in the present work varied between leaf types, from 0.1 mg oxygen/g/h for old kauri leaves to 1.1 mg/g/h for young kanono. Maximum silver beech respiration rates were fairly closely similar to Rounick and Winterbourn's mountain beech, ranging from 0.14 mg/g/h in Reid's Ck in winter, to 0.30 mg/g/h in Hakarimata in summer. These peak rates appeared at 4 -12 weeks in the first run and 4 -16 weeks in the second run, independent of leaf type or stream.

Kjøller and Struwe (1982) summarised the range of mycelial content measurements obtained in randomly collected decomposing leaves in temperate terrestrial habitats (L, F, and H soil layers) as 589 -26000 m mycelium/g. The maximum values found on kamahi and beech in the leaf bag experiment might be expected to be slightly higher than these, as a major limiting factor in soils, water, ceases to be important in streams, although other factors such as aeration may increase in importance. The measured maximum stainable mycelial content ranged from 4955 m/g (beech, Reid's Ck) to 49189 m/g (kamahi, Hakarimata). The values obtained in the present work are only for one group of fungi, the aquatic hyphomycetes. The frequency of isolation data from the plating experiment and the results of the selective inhibition of respiration technique indicate that a somewhat smaller biomass of other fungal groups probably occurred. This was not measured but was responsible for about 30 % of total fungal respiration. Fungal biomass in streams has not been measured very frequently even in overseas studies, and not at all in New Zealand streams. Bärlocher and Kendrick (1974)

found a peak in average mycelial content of 1870 m/g on maple after 36 days. This is low compared to the levels found in the present work. The difference may be a reflection of the difficulty of measuring hyphal lengths directly in leaves.

Spore production values measure the mycelial biomass of aquatic hyphomycetes, but are only comparable when measured under similar conditions, and may vary up to 100 times simply due to differences in species composition. Bärlocher (1982a) recorded up to 6000 x 10^3 spores/g from oak and larch, and 2000 x 10^3 spores/g from spruce, measured over 2 days at 12 C in 200 ml of aerated water. Assuming spore loss rates are the same whether the water is aerated or shaken (4.3.5), these results should be similar to those obtained in the present leaf bag study. Closely comparable results were recorded from kamahi and beech in Hakarimata, while Reid's Ck spore numbers were almost a power of 10 lower. Thus, despite the lower mycelial content found in one northern hemisphere study (Bärlocher and Kendrick, 1974), in another study (Bärlocher, 1982a), aquatic hyphomycete biomass, as measured by spore production, was closely similar to that found in New Zealand in the present study.

In a previous study (Davis and Winterbourn, 1977) of mountain beech in a South Island stream only very poor colonisation by fungi, particularly aquatic hyphomycetes, was detected using a clearing and staining technique, SEM, and by examining the foam produced by bubbling air through water containing leaf samples. These methods would not be particularly effective at detecting and measuring aquatic hyphomycetes compared with measuring spore production as in the present work. Their suggestion that the "leaves of Fagaceae in general may represent relatively poor substrates for aquatic hyphomycetes" is not borne out in the present work, where silver beech was colonised as well as any leaf type in the leaf bag

experiment. In addition, where mountain beech leaves were sampled in the random leaf collections from Reid's Ck, and from other streams in the stream survey, aquatic hyphomycete spore production was only slightly less than that obtained from silver beech in the leaf bag experiment (average from random litter from Reid's Ck compared with average from leaf bag beech in Reid's Ck). In fact, microbial colonisation of any kind appeared to be low in Davis and Winterbourn's stream. In their study mountain beech leaves of various ages removed from the stream and incubated in nutrient enriched or sterile water for one month at 6 or 16 C showed no measureable change in weight. Davis and Winterbourn concluded that microorganisms alone were ineffective as decomposers. This contrasts with the 23 % weight loss from sterile silver beech leaves achieved by aquatic hyphomycete cultures in 77 days (2.5 months at 13 C), and is more like that found for ponga in the present work. It is possible that, like ponga, mountain beech leaves could be colonised by aquatic hyphomycetes, but without causing as much weight loss as on kamahi, for example, through the degree to which the middle lamellae are attacked, resulting in fragmentation of the leaves, compared with the utilisation of less pivotal structural zones (weight respired).

Invertebrate numbers recorded per bag varied in overseas studies depending on the species composition of the fauna. Rosset et al (1982) counted up to 200 -250 individuals per 5 g bag where the leaf shredding amphipod *Gammarus* was dominant, but only about 70 animals per bag where insect larvae dominated the fauna. Davis and Winterbourn (1977) recorded a peak in the numbers of invertebrates colonising mesh bags containing 5 -7 g mountain beech of about 36 animals per bag after 20 days. Rounick and Winterbourn (1983a) found up to 32 animals per bag in a subsequent study in the same

stream, but only 15 in a less stable stream.

The results for the present leaf bag experiment vary considerably, probably in part due to the superficial method of sampling, the peak in numbers ranging from 13 to 54 animals per bag for kamahi and 10 to 32 for beech, independent of the stream. These numbers are, on average, not very different from Davis and Winterbourn's Middle Bush stream numbers, the more conspicuous difference being the time taken to reach them. In the present work peak numbers were recorded after 3 to 12 months depending on leaf type, but all considerably later than the 20 days found by Davis and Winterbourn (1977).

Collier and Winterbourn (1986) found invertebrate numbers peaked at 1.5 -2 months on willow leaves, corresponding to 55 -62 % weight loss. Combining the results from other studies, they suggested that invertebrate numbers reached a peak after a certain amount of decay, constant for each leaf type, for example 10 % for *Acer*, 52 % for *Nothofagus fusca*, 55 -65 % for willow, while numbers increase throughout decay on *Alnus*. In the present work, invertebrates reached a peak in numbers on kamahi at 35 -55 % weight loss (if the odd result of the first run in Reid's Ck was ignored). Beech reached a peak at 40 -45 % weight loss, kauri at 50 -70 %, and kanono at 90 %, although more frequent sampling of kauri and kanono would be desirable.

Overseas studies have varied in the contribution to leaf weight loss attributed to invertebrates. For example, Mathews and Kowalczewski (1969) found leaves exposed in the River Thames in coarse mesh bags lost weight at the same rate as in fine mesh bags from which invertebrates were excluded. This was probably because leaf consuming invertebrates, particularly shredders, are not numerous in large rivers. In contrast, Petersen and Cummins (1974)

found excluding invertebrates from their experimental stream (modelling a small woodland stream) indicated that about 21 % of the leaf weight loss was due to invertebrate consumption.

New Zealand streams appear to be dominated by collector -browser feeding categories of invertebrates, while shredders, the major category responsible for weight loss of allochthonous leaves in northern hemisphere studies, are frequently absent, apart from a few species, e.g. *Zelandopsyche ingens* in some southern beech forest streams (Winterbourn and Davis, 1976; Winterbourn, 1982; Towns, 1976). However, Winterbourn et al (1981) indicated that feeding categories in New Zealand streams tend to be loose, with, for example, several surface-browsing stoneflies and caddises being capable of acting as opportunistic shredders. The pattern seen in the present work, of increased invertebrate numbers corresponding to a certain amount of weight loss (that is state of decay or microbial colonisation) rather than simply length of time immersed, suggests that the leaves were not functioning simply as physical substrates in which to hide, or on which organic layers accumulated as food. In addition, the visual pattern of loss of leaf area in the leaf bag experiment suggested chewing as well as fragmentation was an important source of weight loss.

Winterbourn et al (1981) suggested that "biological communities centred around the direct utilisation of CPOM [Coarse Particulate Organic Matter] energy sources can rarely exist" in New Zealand streams. This was based mainly on the observations that river systems in New Zealand are frequently physically unstable, flood -prone, and therefore not very retentive of leaf litter. It is perhaps as a result of this that shredder feeding categories are so poorly developed in New Zealand. "For shredders to occur, a stream must be able to retain CPOM, as often happens in small, low gradient

tributaries where even in New Zealand shredders can be common" (Winterbourn et al, 1981).

Cummins (1974) has suggested that measurements such as respiration, ATP, or spore production could be used as an indicator of the amount of in-stream processing occurring, or of how retentive a stream is, by comparison with reference standards incubated in the stream for known periods. Suitable standards were obtained in the leaf bag experiment from the spore production measurements. The spore production values obtained from randomly collected leaves in the Hakarimata stream correspond most closely to values for kamahi following two months immersion and processing, that is 162 -3698 x 10^3 spores/g/day for random leaves compared with:

2 months: 269 -365 x 10^3 spores/g/day

3 months: 1230 -5513 x 10^3 spores/g/day

In Reid's Ck they compare well with beech leaves following 2 -3 months immersion, that is 34 -543 x 10^3 spores/g/day compared with:

2 months: 41 -60 x 10^3 spores/g/day

3 months: 255 -637 x 10^3 spoes/g/day

In other words, the average colonisation of collections of leaves of obviously varied states of decay was frequently less than the peak colonisation recorded in the leaf bag experiment, but high enough to suggest that a good proportion of the leaves being retained were retained long enough to reach peak colonisation in the Hakarimata stream and Reid's Ck.

In the stream survey only leaf samples from one of the alpine streams showed markedly lower spore productivity than that seen in the range from either Hakarimata or Reid's Ck. This suggests that collectable litter is retained litter, and that where litter is retained in any of the stream types sampled it becomes colonised and presumably degraded in a fashion not dissimilar from that in

Hakarimata or Reid's Ck.

However, the surveyed streams differed in the ease with which a litter sample could be collected, that is, how much litter enters the stream and is retained, and this was reflected by the spore concentration in the water, measured by filtering samples of stream water directly. Generally, relatively lowland streams (up to an altitude of 350 -400 m) had 1,000 to 9,000 spores/l, compared to fewer than 1,000 spores/l in streams at higher altitudes. Alpine streams clearly had the least resident litter and hence the lowest spore concentrations. Thus filtering a water sample and counting the aquatic hyphomycete spores present has the potential to measure the stability of any unknown stream, or in general terms, to measure the occurrence of conditioned litter habitats suitable for invertebrates.

As noted by Aimer and Segedin (1985a) spore concentrations in forested lowland streams in New Zealand compare well with those measured in temperate North American and European studies (Bärlocher and Rosset, 1981; Iqbal and Webster, 1973; Michaelides and Kendrick, 1978; Wood-Eggenschwiler and Bärlocher, 1983). This suggests that retained litter levels are similar.

In general terms, the results for respiration, mycelial content, and aquatic hyphomycete spore production found in the Hakarimata stream and Reid's Ck were comparable to those found in the northern hemisphere, where the work elucidating the interrelationship between allochthonous leaf litter, microbial conditioning, and invertebrate consumption has been carried out (Kaushik and Hynes, 1968, 1971; Petersen and Cummins, 1974; Bärlocher and Kendrick, 1981). The potential for the same kind of detrital cycle, involving aquatic hyphomycetes, obviously exists in some New Zealand streams at least, perhaps with slightly less participation from invertebrates than

found in the studies mentioned above.

Thus the present work, although not measuring directly the importance of CPOM and its decomposition to energy transfer in the whole stream ecosystem, indicates that the potential for its contribution in many streams is equivalent to that demonstrated or hypothesised in many northern hemisphere studies (Fisher and Likens, 1973; Minshall et al, 1983). While organic layers on stone surfaces undoubtedly have an important role as sites of energy transfer in many New Zealand streams (Winterbourn et al, 1981; Winterbourn, 1985), particularly where resident litter levels are low, such as fast flowing upland streams, and perhaps lowland streams in pasture habitats, the role of the CPOM or detrital cycle should not be ignored, particularly in North Island streams in forested catchments.

8.2 CHANGES IN AQUATIC HYPHOMYCETE SPECIES DURING LEAF DECAY: SUCCESSIONAL PATTERNS.

8.2.1 Comparison with Previous Work.

In the present work a sequence of at least 3 groups of fungi was found in the leaf bag experiment, each dominating different stages of leaf decay):

1)EARLY DECAY: *F. penicillioides*, *H. lugdunensis*.

2)MID DECAY: *Lunulospora* spp., *L. ovalis* sp. ined., *A. filiformis*, *A. pulchella*, *A. tetracladia*, *T. chaetocladium*, *T. elegans*, *T. marchalianum*, and probably Unidentified s/r type 1(2). In the

absence of early decay species *A. filiformis* appeared to be the fastest colonising species, while *T. chaetocladium*, *A. pulchella*, and Unidentified s/r type 1(2) showed a tendency to reach peak spore production slightly later.

3)LATE DECAY: *A. acuminata*, *A. longissima*, *C. aquatica*, *F. eccentrica*, *L. aquatica*, and Unidentified s/r type 1(3).

The same pattern was repeated in both streams, seasons, and on most leaf types, although there was some variation in the actual species between streams and seasons.

Previous studies which have followed the aquatic hyphomycetes present during leaf decomposition have differed as to whether a successional pattern appeared or not. Where one did occur the fungal flora was not always the same as that of the leaf bag experiment, so that there are relatively few comparisons of the actual time during decay when a particular species occurred. Previously published successions are discussed first, followed by studies without successional patterns and the concept of "founder controlled communities" derived from them.

Six species which showed a successional pattern in the present work were also found in one or more overseas studies. *H. lugdunensis* was most prominent in early leaf decay in two studies (Bärlocher et al, 1978; Bärlocher, 1982a), as in the present one, but in a study by Bärlocher and Kendrick (1974) it was most abundant later in decay. It has also been found frequently on decaying wood and twigs, sometimes apparently as an early colonising species (Webster and Hawksworth, 1986).

T. marchalianum and *L. aquatica* were mid decay and probably late decay species respectively in the present work, but rather variable in previous studies. Both were recorded as more abundant in early decay once, mid decay once, and *T. marchalianum* as a late decay

species once (Bärlocher and Kendrick, 1974; Suberkropp and Klug, 1976, 1981; and Bärlocher et al, 1978).

A. acuminata, *C. aquatica*, and *A. longissima* were late decay species in the present study. Three previous studies found the same pattern in the latter two. Five studies found the same pattern in *A. acuminata*, although in two it was rather variable (Bärlocher and Kendrick, 1974; Bärlocher et al, 1978, Bärlocher, 1982a; Suberkropp and Klug, 1976, 1981).

Thus previous studies showed a fairly good correspondence with the present work in four out of six species, despite differences in leaf type and stream ecology (water chemistry, invertebrate species etc.). This suggests that at least some of these successional patterns result from stable, real differences in the ecology of the species concerned, and that aquatic hyphomycetes are behaving in a closely similar fashion in New Zealand streams and northern hemisphere streams.

A number of studies, however, did not find a succession of species during decay, for a variety of reasons. Chamier (1987) stated that the double peak in total spore numbers versus time found on oak leaves resulted from a succession of fungal species. The same successional pattern did not occur on the faster decaying alder leaves in the same study, rather like hangehange in the present study. Thus successional patterns may not appear on quickly processed leaves. Bärlocher (1982a and b) suggested that successional patterns were more common in the presence of invertebrate detritivores than when they were excluded by fine mesh bags. Studies by Chamier and Dixon (1982), Bärlocher and Schweizer (1983), and, apparently, Padgett (1976) also failed to find clear

successional patterns among aquatic hyphomycetes on decaying leaves.

Bärlocher (1982a) concluded that the aquatic hyphomycete flora of decaying leaves normally consists of 4 -8 common species which arrive early and persist throughout decay, together with a number of rarer species that arrive later and vary from sample to sample, apparently unpredictably. Bärlocher and Schweizer (1983) found a significant correlation between the time of arrival of a species and its total spore production throughout decay. The data from the present work can be arranged to show the number of species appearing for the first time in each sample, grouped according to the average spore production/g during leaf decay (Table 8.4).

Table 8.4: Number of species appearing for the first time in each sample from kamahi (sampled after 1, 2 weeks, then monthly), grouped according to the average spore production/g during leaf decay into >10 spore/g, 1 to 10 spores/g, and <1 spore/g. The results are given separately, for Hakarimata first and then second run kamahi, followed by Reid's Ck first and then second run kamahi.

	Time of first recording, in weeks													
	1	2	4	8	12	16	20	24	28	32	36	40	44	48
HAKARIMATA														
Kamahi, 1st run														
> 10 spores/g:		1	3	2	4									
1 -10 spores/g:			1	1	2	1								
< 1 spore/g:	1	2	1		2	1	1							
Kamahi, 2nd run														
> 10 spores/g:		1	3	2										
1 -10 spores/g:				4	1									
< 1 spores/g:			2	3			1	1	2	1		1		1

Table 8.4 cont.

	Time of first recording, in weeks													
	1	2	4	8	12	16	20	24	28	32	36	40	44	48
REID'S CK														
Kamahi, 1st run:														
> 10 spores/g:			3	1	5		1							
1 -10 spores/g:			1	1	5		1	1						
< 10 spores/g:			2		4		2		1					
Kamahi, 2nd run:														
> 10 spores/g:			5	3										
1 -10 spores/g:			2	2	2	1			1					
< 1 spores/g:			5	2	2	1		1						

Similar patterns appeared in the results for beech.

All the major species appeared early on in both seasons and streams. The first appearances of the less important species were spread over a larger number of samples, but the number of first appearances with time tended to reach a peak about the same time as that for the more abundant species. There was no consistent pattern in which most of the less important species arrived later, as would be expected if the time of arrival was the reason for the lower average spore numbers. This suggests that from the first month on at least the time of first appearance of a species was a sampling phenomenon rather than a colonisation phenomenon, and that the same pattern of average abundance relative to time of first appearance would be visible in the data for randomly collected leaves when treated in the same manner (Table 8.5).

Table 8.5: Number of species appearing for the first time in each sample from random leaf samples (sampled monthly), grouped according to the average spore productivity/g into >10 spores/g, 1 to 10 spores/g, and <1 spore/g. Hakarimata and Reid's Ck results are given separately.

	Time of first recording, in weeks.											
	4	8	12	16	20	24	28	32	36	40	44	48
HAKARIMATA												
> 10 spores/g:	4											
1 -10 spores/g:	6	1										
< 1 spore/g:	2				1	1		1	1	1	1	1
REID'S CK												
> 10 spores/g:	5											
1 -10 spores/g:	5	3	1									
< 1 spores/g:	4	3	4		1		2			1	1	

The results for kamahi differ from these randomly collected leaves only in the slight initial lag in the appearance of all species, and in the continued appearance of rare species being limited by decay consuming the substrate. Thus the late appearance of some less important species in the leaf bag experiment probably relates to seasonal and sampling factors. This result, together with the clear appearance of a successional pattern among the dominant species, does not support Bärlocher's (1982a) idea of a founder controlled community, at least in the New Zealand streams studied here.

8.2.2 Causal Factors.

Differences in the rate of colonisation and growth offer the simplest explanation of why some species were slower to increase in

spore numbers than others. All the major species appear at about the same time, but rates of subsequent growth could have differed. Yet from the present work (5.3.1), Koske and Duncan (1974), and Arsuffi and Suberkropp (1984) it is evident that growth rate could have influenced the sequence of attainment of peak numbers in only a few species, such as *A. acuminata*. Thus differences in growth rates would not in themselves explain the sequential decline of species.

The present work has measured the average occurrence of aquatic hyphomycetes on whole leaves. It is possible that different tissue types within the leaf may have been preferred by different species, in which case the different rates at which the tissue types were colonised and decayed could result in an apparent successional pattern between species. Bärlocher and Kendrick (1981) showed that *T. angulatum* was more common on the lamina, and *H. lugdunensis* on the veins and stalks of leaves. As the lamina was consumed first *T. angulatum* declined while *H. lugdunensis* continued to increase in abundance as decay proceeded. *H. lugdunensis* appeared as an early decay species in the present work, suggesting, if anything, the reverse distribution, i.e. reflecting a preference for the most rapidly decayed tissue. Chamier et al (1984) found that the ribs of alder leaves were colonised more slowly than the lamina, but no difference in the species composition of the two tissues was mentioned. No successional pattern was found on alder, despite the selective disappearance of different tissue types (Chamier, 1987). Shearer and Lane (1983) also mapped the distribution of species on whole leaves (excluding petioles), but did not mention any difference between leaf veins and lamina. Thus it would appear that preferences for different tissue types were unlikely to have been an

important cause of the successional pattern observed in the present work.

It has been demonstrated in overseas work that leaf shredding invertebrates consume leaves colonised by different aquatic hyphomycetes selectively, and that the order of preference changes with the length of time the fungi have been growing on the leaves (Bärlocher, 1985; Suberkropp et al, 1983; Arsuffi and Suberkropp, 1984, 1985; Butler and Suberkropp, 1986). Thus it is possible that the sequence of species observed in the successional pattern represents a sequence of most immediately palatable to the slowest to become palatable (or least palatable). This would explain the decline of each group prior to the next, although not the sequential increase in abundance (actual spore numbers). The only information on preferential feeding comes from northern hemisphere studies, and hence involves different invertebrates feeding on different leaf types.

However, from the work of Butler and Suberkropp (1986), Arsuffi and Suberkropp (1984, 1985) it can be suggested that in the present leaf bag experiment *A. acuminata* was likely to have been a highly palatable species, but not until after some length of time, during which *T. marchalianum*, *L. aquatica*, *C. aquatica*, *T. elegans*, and *H. lugdunensis* would have been preferred, though in varying degrees. Thus selective feeding by invertebrates might have had some effect on the occurrence of *A. acuminata* as a late decay species compared with *H. lugdunensis*, *T. marchalianum* and *T. elegans*, but there is insufficient evidence to separate the other species.

"Modification of the habitat is probably the principal driving force of a succession" (Frankland, 1981). A number of factors

change during leaf decay: physical structure, levels of tannins and inhibitory byproducts, pH, and the nutritional composition of the substrate. Triska (1970) suggested pH as a possible factor in succession as it often rises with decay (Sjörs, 1959). All the species tested by Rosset and Bärlocher (1985a) had a pH optimum for growth of 4 -5. The early decay species (in the present work) *H. lugdunensis* showed the best growth at higher pHs (7 -8), closely followed by *T. marchalianum*, a mid decay species. In comparison, *A. acuminata*, *C. aquatica* (late decay species), and *T. elegans* (a mid decay species) ceased growth above 7. Thus pH does not appear to be a factor in the successional patterns observed, at least among the species tested by Rosset and Bärlocher (1985a).

The traditional idea of a fungal succession reflecting a similar stepwise utilization of the components of the substrate has for some time been considered too simple. There is little evidence of any species in a succession dying out from starvation: the ability to compete on a substrate rather than simply utilize it is more important (Frankland, 1981). Certainly there is no correlation between the position in the successional pattern determined in the present work and the species' ability to utilize either cellulose (Suberkropp and Klug, 1981; Chamier, 1985) or lignin (Fisher et al, 1983). There is, however, little information available on how well different aquatic hyphomycetes compete with each other on different substrates.

Competitive interactions have been suggested as a possible source of successional patterns (Wicklow, 1981), in which a "competitive hierarchy" may be seen to occur. There has been some previous work done on the competitive interactions between aquatic hyphomycetes. Leaves inoculated with one of six species of aquatic

hyphomycete and exposed in a stream resisted invasion by other species to a greater or lesser extent (Rosset and Bärlocher, 1985b), suggesting competitive interactions of some sort may exist among aquatic hyphomycetes. Chamier et al (1984), in explaining the dominance of alder leaves by a small group of species, suggested that the initial colonisers created areas which were more readily colonised by a limited number of compatible species through the inhibition of other species. Shearer and Zaire-Maivan (1988) tested *in vitro* hyphal interactions among wood and leaf inhabiting fungi from fresh water, including some aquatic hyphomycetes. Generally, species found on wood, or wood and leaves, inhibited other species more and were themselves less inhibited by other species, compared with those found on leaves alone. Early colonists on wood appeared to be less aggressive than later colonists. Only four of the aquatic hyphomycetes tested were abundant in the present work. These were, in order of decreasing interaction indices (sum of species rankings according to degree of inhibition plus resistance to inhibition): *C. aquatica* (wood and leaves, late decay on leaves in the present work), *T. marchalianum* (wood and leaves, mid decay on leaves in the present work), *H. lugdunensis* (wood and leaves, early decay on both), *C. longibrachiata* (leaves only, mid or late decay in the present work). With the exception of *C. longibrachiata* then, there is a correlation between the competitive hierarchy and successional pattern. Thus interference competition may be involved in the successional patterns observed in the present work.

In conclusion, a number of factors might have been important in determining the successional pattern seen among aquatic hyphomycetes in the leaf bag experiment. Growth rates, palatability to

invertebrates, or competitive ability, perhaps on different substrates, may have been involved. The latter seems most likely, as it could explain the variation in abundance of different successional groups on some leaf types, most notably the dominance of ponga by late decay species at the expense of mid decay species. Thus certain leaf types correspond to stages of decay on others, the common factor presumably being the biochemistry of the substrate, acting through the ability of late decay species to compete with other microorganisms on it.

8.3 DIFFERENCES IN COLONISATION BETWEEN LEAF TYPES.

8.3.1 Number of Species.

Bärlocher (1980) showed that there was no difference in peak number of species between medium and slow decaying leaf types, only the rate at which the peak was attained. In contrast, fast decaying leaves, or those with a shortened residence time for whatever reason, were colonised by fewer species, the late, rare species, in particular, being absent (Bärlocher, 1980; Rossi, 1985).

The present data also showed this pattern. Kauri, much slower decaying than kamahi, had fewer species in the first 2 months, but the same peak number of species. Rimu, also slower, showed the same peak number of species, although no early samples were taken to enable determination of the rate of increase in number. In contrast, hangehange, the fastest decaying leaf type, initially had more species than kamahi, but the peak number of species was only about half that on kamahi. Young kanono, which also decayed faster than kamahi, initially differed little, but the peak number of

species was conspicuously lower. Dried kamahi decayed faster than fresh kamahi and fewer species appeared, although because a greater number of spores per g were produced fewer fields on the filters were counted, which may have reduced the number of species seen.

Bengtsson (1982) found northern hemisphere beech (*Fagus*) was colonised by markedly fewer species of aquatic hyphomycetes (3) than other broadleaf types, for example, alder (12). In the present work only very slightly lower numbers of species were found on the southern hemisphere beech (*Nothofagus*) compared to kamahi. Possibly the experimental period used by Bengtsson was too short to allow full colonisation of beech, which is a much slower decaying leaf type than alder. The alternative is that *Fagus* and *Nothofagus* differ markedly in some respect important to fungi, but this is difficult to envisage. Another New Zealand *Nothofagus* species (mountain beech) appeared to be poorly colonised by aquatic hyphomycetes in the study by Davis and Winterbourn (1977). This also was probably the result of the method of measuring spore production. Gönczöl (1975) depicted well decayed beech (*Fagus*) leaves that were heavily colonised by aquatic hyphomycetes. Nilsson (1964) observed that aquatic hyphomycetes were commonest on alder and several other leaf types, less common on beech and birch, and rare on very hairy leaf types such as elm, also suggesting beech was not exceptionally different from other leaf types.

8.3.2 Spore Productivity of Aquatic Hyphomycetes in Different Leaf Types.

In the present work a similar relationship existed between total and average spore production from different leaf types and rate of

decay as between number of species and rate of decay, with the exception of dried kamahi (Tables 5.7 and 5.7a). Thus fast-decaying hangehange and young kanono had much lower spore production values than kamahi, beech, or kauri. Few aquatic hyphomycete spores were produced from a randomly collected sample of *Egeria densa* (Waipa River) which, like other thin-leaved macrophytes (Bird and Kaushik, 1985), decayed very rapidly. Dried kamahi differed conspicuously in that it lost weight at a similar rate to hangehange in Hakarimata in summer, but was more heavily colonised by aquatic hyphomycetes than fresh kamahi. The pattern of decay also differed from that of other fast decaying leaf types, being brown in colour, less soft and mushy, and more rapidly fragmented and chewed. This suggested that decay pattern, while frequently associated with decay rate, was the more important determining factor with regard to aquatic hyphomycete colonisation.

In contrast to the soft-rotting leaf types, all the more rigid, slow-decaying leaf types sampled regularly (fresh kamahi, beech, and kauri) had roughly similar peak and average spore numbers, the only difference being the initial lag in kauri compared to kamahi. Nilsson (1964) also noticed that different rates and patterns of leaf decay were associated with differences in abundance of aquatic hyphomycetes. Aquatic hyphomycetes were generally rare on leaves that were very slimy or soft rotting. Bärlocher and Kendrick (1981) suggested that high-food-quality leaf types would support faster growth of aquatic hyphomycetes, but would be consumed by invertebrates before they could be as highly colonised as less palatable species.

Tawa, nikau, rimu, and ponga were also slow decaying leaf types, but were sampled less regularly. Tawa and rimu gave rise to spore production figures of a similar magnitude to kamahi, indicating they

were not very much less acceptable as substrates to aquatic hyphomycetes.

Kauri and rimu thus behaved much like other gymnosperms examined in overseas studies, being slowly colonised at first compared to broadleaf types, but eventually being almost as well colonised (Bärlocher et al, 1979; Bärlocher, 1980). Bärlocher et al (1979) attributed the lag in colonisation of *Pinus leucodermis* needles to high levels of biochemical inhibitors (including phenols) which are lost only slowly, due to well developed, highly lipophilic outer layers (cuticle and associated waxes etc). The same mechanism probably operated in the case of kauri and rimu.

In contrast, nikau showed distinctly lower spore production figures than kamahi after comparable weight losses. There has been little previous work on monocotyledons in relation to colonisation by aquatic hyphomycetes. Chamier (1987) found a moorland grass gave rise to much lower spore counts than oak or alder. Bird and Kaushik (1987) found a grass to be slower decaying than either a fern or maple leaves, and quoted Michaelides (pers. comm.) that "fungi were sparse on the surface of ferns and grasses, whereas bacteria were numerous". Possibly monocots in general may be relatively poor substrates for aquatic hyphomycetes. The conspicuous presence of several ascomycetes on nikau suggests a quite different fungal flora may dominate its decay, and perhaps that of other monocots.

Spore production recorded from ponga was also markedly lower than that from kamahi. Total spore numbers from ponga after 6 months (25 % weight loss) were fairly comparable to those from kamahi at 25 % weight loss, although the aquatic hyphomycete flora differed considerably. However, spore production was much lower at 12 months (59 % weight loss). Aimer (1982) found levels of spore production from the fern *Blechnum capense* which were comparable

throughout decay to those from several broadleaf species including kanono, and markedly greater than those from hangehange. However, the method used to incubate the leaves reduced sporulation generally and therefore may have reduced the differences between leaf types in Aimer's study. In addition, only the lamina was incubated (as leaf discs), whereas more of the tough, and possibly poorly colonised rachilla was sampled in the present study. The lamina of ponga differs from *B. capense* in being still more sclerophyllous, and more finely divided, thus tending to trap more silt and algae, which may have inhibited the fungi. Even allowing for a larger proportion of the sample weight remaining to be composed of rachilla, ponga is still clearly not as well colonised by aquatic hyhomycetes as kamahi, but is nowhere near as poor a substrate as nikau, for example.

8.4 DISTRIBUTION OF AQUATIC HYPHOMYCETES BETWEEN STREAMS AND SEASONS.

8.4.1 Seasonal Patterns in Total Spore Concentration.

A pattern of high concentrations of aquatic hyphomycete spores in stream water in summer, and lower numbers in winter, occurred in Hakarimata and Reid's Ck. In a previous study in a New Zealand stream (Nihotupu stream, Auckland) Aimer and Segedin (1985a) found a peak in spore concentration occurred in autumn and winter. They and other workers (e.g. Iqbal and Webster, 1973, 1977) have related such seasonal patterns to the pattern of litter input from seasonal litterfall. If litter levels are the prime factor then the litterfall of the vegetation surrounding Hakarimata and Reid's Ck

obviously must have followed a different pattern from that surrounding the Nihotupu.

The pattern of seasonal periodicity in spore numbers described by Aimer and Segedin (1985a) reflected the litterfall study (Silvester, unpublished) of sites in the same area as the Nihotupu Stream better than the studies from more southerly podocarp -broadleaf sites. Daniel (1975), Daniel and Adams (1984), Levett et al (1985), and Cowan et al (1985) found southerly podocarp -hardwood forests to show a summer peak in litter production, with high levels occurring from November till March or later. Allowing a residence time of 1 -2 months on average (estimated from the amount of fungal colonisation of randomly collected leaves in the present work), litter levels in streams flowing through this vegetation type might be expected to be high throughout summer and autumn. This pattern fits the seasonal pattern in spore numbers found in the Hakarimata stream well.

The riparian vegetation surrounding Reid's Ck was dominated by mountain and silver beech. There is no information on litter patterns of silver beech, but Wardle (1970) found peak litterfall of mountain beech occurred in December and again in March -May. Bagnall (1972) found a similar pattern for hard beech: a spring peak associated with new leaf development, and a green leaf peak in autumn associated with storm losses. Thus it is likely that litter levels in Reid's Ck increased in summer, and again in autumn or early winter, to a lesser extent. The spore numbers in Reid's Ck clearly followed the first peak in litterfall, but there was no sign of an autumn increase.

Litter levels in a stream may be influenced by lateral transport of terrestrial litter into the stream (presumably not highly seasonal), and seasonal discharge patterns, in addition to patterns

of litterfall. The amount of seasonal variation in discharge and its effect on litter retention in the stream will depend on the stream type; for example Winterbourn (1976) found discharge had a governing effect on litter levels in a South Island beech stream, whereas litterfall patterns (combined with a lengthy retention time) clearly were more important in the stream studied by Aimer and Segedin (1985a), as in Hakarimata and Reid's Ck in the present work.

Akridge and Koehn (1987) studied a constant temperature river (22 C) in Texas, and found a pattern of high numbers of spores/L, apparently in response to levels of deciduous leaf litter alone. However, factors other than increased litter levels due to litterfall in summer may have contributed to higher numbers of spores in Hakarimata and Reid's Ck: fewer floods washing leaves away before colonisation, higher temperatures, and perhaps higher dissolved nutrient levels in the stream water.

Most aquatic hyphomycetes have *in vitro* optimum temperatures closer to the range seen in Hakarimata and Reid's Ck in summer than winter, that is 15 -25 C (present study; Koske and Duncan, 1974; Suberkropp, 1984). It would appear that optimum litter levels and temperature coincide in New Zealand streams like Hakarimata and Reid's Ck, unlike more northerly streams such as the Nihotupu, and most temperate northern hemisphere streams (Bärlocher and Rosset, 1981; Del Frate and Caretta, 1988; Iqbal and Webster, 1973, 1977; Müller-Haekel and Marvanová, 1979). This ought to allow aquatic hyphomycetes to play an even greater role in litter decomposition in New Zealand streams than in northern hemisphere streams. However, the highest mycelial content calculated for kamahi and beech leaves in the leaf bag experiment, in both Hakarimata and Reid's Ck, occurred in winter. Thus the seasonal pattern of aquatic hyphomycete mycelial content in leaf litter appeared to be the

reverse of that of spore concentrations in stream water. Aimer (1982) found some evidence of a similar pattern in spore production from leaf bag leaves when measured at a constant temperature, that is, higher production in winter compared with summer.

The most likely explanation is that the higher, summer temperatures also favour other fungi and bacteria, which compete with aquatic hyphomycetes for the leaf litter. In particular, if oxygen concentrations fall in response to increased biological activity, then aquatic hyphomycete colonisation would be expected to be inhibited (5.3.2 and 5.3.3). It is possible that the leaf bag method of presentation in the stream predisposed the leaf litter to oxygen deprivation. There did not appear to be any seasonal pattern in spore production or mycelial content in the randomly collected litter. It is not clear therefore how much impact seasonal patterns of mycelial activity could have on naturally occurring leaf litter, but it would appear to be small.

8.4.2 Seasonal and Distributional Patterns in Individual Species: Comparison with Previous Work.

In the present work different seasonal preferences were observed in different species, apparent in both the number of conidia/l of stream water, and in the colonisation of first compared with second run leaves in the leaf bag experiment (Table 8.6). In a number of cases these preferences differed between Hakarimata and Reid's Ck, and in a predictable fashion. Thus there was a clear connection between seasonal distribution and distribution between streams which was continued in the stream survey. Species which were more abundant in winter than summer in Hakarimata were generally more

abundant in Reid's Ck than Hakarimata, and in beech streams compared with podocarp -hardwood streams generally. Species abundant in both Reid's Ck and Hakarimata frequently showed a shift in seasonal abundance from winter maximum in Hakarimata towards summer maximum in Reid's Ck. Species more abundant in winter in Reid's Ck were frequently low or absent from Hakarimata and other podocarp -hardwood streams. Some species which were relatively rare in both beech and podocarp -hardwood streams, or more common in beech streams, were most abundant in alpine streams.

Thus the major species in the present work could be divided into four groups, on the basis of their seasonal periodicity and distribution, as follows:

1) Species tending to be more abundant in summer than winter in Hakarimata and absent or similarly more abundant in summer in Reid's Ck, and which were more abundant in podocarp -hardwood than beech: *E. microaquatica*, *F. penicillioides*, *Lunulospora* spp., Unidentified sp. 1, and probably *T. marchalianum*.

2) Species generally more abundant in winter in Hakarimata, and tending towards greater abundance in summer in Reid's Ck most commonly did not show a clear difference in distribution between forested stream types: *A. acuminata* (but more abundant in beech streams), *Alatospora* spp., *A. filiformis* (but more abundant in beech streams), *A. longissima/crassa*, *C. aquatica*, *C. longibrachiata*, and probably *T. acuminatus*.

3) Species more abundant in winter in Reid's Ck and very low or absent from Hakarimata, and which were more abundant in beech than podocarp -hardwood streams: *A. tetracladia*, *F. eccentrica*, *T. elegans*, *T. gracilis*, Unidentified s/r type 1(3), and probably *D. aquatica* and Unidentified s/r type 1(2).

Table 8.6: Summary of the seasonal patterns observed among species in the stream filters (in % total spores in Hakarimata and Reid's Ck) and in the leaf bag leaves (in spore production on kamahi and beech leaves in each stream), together with the patterns of distribution in the leaf bag experiment (abundance in Hakarimata compared with Reid's Ck) and in the stream survey (abundance in streams grouped according to vegetation type and latitude). The minimum and optimum temperatures for mycelial growth (colony radial extension on agar in pure culture) determined in the present work and in previous work (Suberkropp, 1984) are also given.

	SEASONAL PATTERNS						DISTRIBUTION		GROWTH TEMP. (C)			
	Str. filters		Leaf bag lvs.				Lf bag Str. survey		Present		Previous	
	Hak.	Reid	H/ka	H/be	R/ka	R/be			Min.	Opt.	Min.	Opt.
A. acuminata	W?	NP	NP	W	S	NP	R	A>B>others			<1	20
Alatospora spp.	NP	NP	W	NP	(NP?)	NP	NP?	NP				
A. crassa/longiss.	NP	NP	W	NP	(NP?)		NP	NP?	<5	25	<1	15 -25
A. filiformis		S?	W	W	W	S?	R	SPH+B>NPH	1	25		
A. tetracladia		(W?)			W?	NP	R	A>B>?SPH>?NPH	<1	20	<5	20 -25
C. aquatica	NP?	Spr	W	W	S	NP	NP	NP	1	25	<1	25 -30
C. longibrachiata	NP?	NP?	W	W	S	W	R?	A>others				
D. aquatica					(NP?)	(W?)						

Table 8.6 cont.

	SEASONAL PATTERNS							DISTRIBUTION	GROWTH TEMP. (C)			
	Str. filters		Leaf bag lvs.				Lf bag	Str. survey	Present		Previous	
	Hak.	Reid	H/ka	H/be	R/ka	R/be			Min.	Opt.	Min.	Opt.
E. microaquatica			(S)	(W)			H?					
F. penicillioides	NP	(NP)	S	S			H?	NPH+SPH>B<A	5	25	5 -10	25 -30
F. eccentrica					W	(W)	R?	A>B+SPH>?NPH				
H. lugdunensis					S	(NP?)	R	B>?others			?	20 -25
L. aquatica					NP		R?	NPH+SPH>?B	1	20-25	<1	20
L. ovalis	NP		W	W			H	NPH>others	>1	25		
Lunulospora spp.	S?	S	W	S	S	S	NP?	NPH+SPH>B>A	>5	20		
T. gracilis		W					R?					
T. elegans		NP			W	W	R	SPH+B>NPH+A				
T. marchalianum	S?		NP?				H?	NPH+SPH>B+A			<1	20
T. chaetocladium	(NP)	(NP)	W?	NP?	NP?	W	H?	NP?	1	20	1	15
T. acuminatus			(S?)	(S?)	(S?)	(S)		NP				
V. elodeae								A>others	<1	20-25	<5	20-25
Unident. sp. 1	S?						H	NPH+SPH>B+A				
Unident. s/r 1(2)	NP	W	(W?)	(NP?)	W	W	R?	B>SPH>NPH+A			<1*	15 -20*

Table 8.6 cont.

	SEASONAL PATTERNS						DISTRIBUTION	GROWTH TEMP. (C)			
	Str. filters		Leaf bag lvs.				Lf bag Str. survey	Present		Previous	
	Hak.	Reid	H/ka	H/be	R/ka	R/be		Min.	Opt.	Min.	Opt.
Unident. s/r 1(3)					NP	NP	R	A+B>SPH+NPH			

*F. curvula

W = winter

H = Hakarimata

< = growth at minimum tested

S = summer

R = Reid's Ck

NP = no seasonal pattern

? = pattern not very clear

() = low numbers

NP = no difference between streams

? = pattern not certain

A = alpine

B = beech

SPH = southern podocarp -hardwood

NPH = northern podocarp -hardwood

Species which did not fit into any of the above patterns (among forested streams) were *H. lugdunensis* (absent from Hakarimata but more abundant in summer in Reid's Ck), *L. ovalis* sp. ined. (a winter species in Hakarimata but absent from Reid's Ck), and *T. chaetocladium* (no clear seasonal or distributional pattern).

4)Species which occurred in alpine streams in abundances in excess of those in other stream types, including beech: *A. tetracladia*, *F. eccentrica*, and *V. elodeae*. Other species were present at levels similar to those in beech streams, and were thus of greater relative importance only in alpine streams (% of total), for example *A. acuminata*, *Alatospora* spp., *C. longibrachiata*, *F. penicillioides*, and perhaps *L. aquatica*, *T. gracilis*, *T. chaetocladium*, Unidentified s/r type 1(3), and Unidentified s/r type 22.

It was possible to distinguish two groups of podocarp -hardwood streams (northern and southern) on the basis of several species which occurred in numbers either like those in Hakarimata or Reid's Ck, for example, *L. ovalis* sp. ined. was generally abundant in Hakarimata and northern podocarp -hardwood streams, but absent from Reid's Ck, beech, and southern podocarp -hardwood streams, while *T. elegans* showed the reverse distribution. The two groups of podocarp -hardwood streams were only distinguisable because there were sufficient streams in the podocarp -hardwood category to subdivide into groups with enough members to give meaningful averages from which trends could be observed. "Beech" and "alpine" stream types may perhaps have contained similar subgroups, but the small number of samples of each prevented trends within these groups being distinguishable, only comparisons between groups being possible.

The seasonal and distributional patterns outlined above can be compared with those in previously published work from streams in a similar climatic zone (Aimer and Segedin, 1985a; Gönczöl, 1975; Shearer and Webster, 1985a and b; Suberkropp, 1984; Triska, 1970).

The seasonal patterns seen in temperate northern hemisphere studies of forested streams, where the same species occurred, correspond surprisingly well to those found in the leaf bag experiment, or to the preference for colder or warmer conditions that seasonal patterns imply. Thus *A. acuminata*, *A. tetracladia*, *L. aquatica*, and Unidentified s/r type 1(2) (if it is *F. curvula*) were fairly consistently more abundant in winter, whereas *F. penicillioides*, *Lunulospora* spp. (*L. curvula* at least), and probably *T. marchalianum* were more abundant in summer in previous work, as in the present work. *C. aquatica* and *C. longibrachiata* showed little or no seasonal variation in previous work, but appeared to be more abundant in winter in Hakarimata in the present work. However, they were relatively widespread species in the present stream survey. Previous studies have differed with regard to *T. elegans*, Triska (1970) recording its presence throughout the year, while Shearer and Webster (1985b), in a short study, found it decreased in importance from October to February (i.e. during autumn and winter). In the present study, however, it was clearly more important in winter and in colder streams.

The study by Aimer and Segedin (1985a) relied on foam sampling, which probably accounts for the differences in relative abundance of several species compared with the present work. Thus *A. acuminata*, *A. tetracladia*, and *T. acuminata*, all small, tetraradiate-spored

species, were commonly abundant or dominant in foam samples, but much less dominant in filter and leaf samples generally, whereas large tetraradiate-spored species such as *T. elegans* and *T. chaetocladium* were present in low numbers in foam samples, as were sigmoid-spored species. For this reason small tetraradiate-spored species appeared to have a wider distribution among stream types in Aimer and Segedin, for example, *V. elodeae*, which in the present work was found only in alpine streams, appeared in forested streams also, though mostly at fairly high altitudes. Similarly, large tetradiate-spored species showed a wider distribution in the present work than in Aimer and Segedin, for example, *T. elegans* and *T. chaetocladium* were common in South Island samples in the present work, but absent in Aimer and Segedin.

The latitudinal division into "northern" and "southern" podocarp -hardwood stream types, on the basis of the distribution of species such as *L. ovalis* sp. ined. and *T. elegans*, affords interesting parallels with the distribution of some higher plant species (Godley, 1975). Kauri and taraire extend south only to about 38° S, as did *L. ovalis* sp. ined. in the present work. Kamahi extends north to about the same point, rather like *T. elegans* in the present work. It seems likely that both fungi and tree species are responding to the same environmental factor, and that it is an abiotic one. Temperature is the obvious common factor, stream factors such as water chemistry being unlikely to affect trees.

Several workers have sampled alpine and moorland streams in similar northern hemisphere latitudes (Nilsson, 1964; Iqbal and Webster, 1977; Shearer and Webster,1985a), and these show distinct similarities in their aquatic hyphomycete floras to the New Zealand

alpine streams sampled in the present work. All found the aquatic hyphomycete communities to be poorer both in abundance and in number of species. Most found *A. acuminata*, *A. tetracladia*, *C. longibrachiata*, *F. eccentrica*, and *V. elodeae* to dominate the flora, sometimes with some other species, notably those specific to moorland grasses and *Juncus* (Iqbal and Webster, 1977).

8.4.3 Effect of Temperature on Distribution and Seasonal Periodicity.

Temperature is an obvious connecting factor between season and stream type. In summer Hakarimata ranged from 10 to 18 C, and in winter from 5 to 12.5 C. In Reid's Ck summer temperatures ranged from 4 to 20 C, and in winter from 0.5 to 8 C. Alpine streams were likely to become as cold or even colder than Reid's Ck and for longer periods. Thus temperature probably increases from alpine to beech to southern podocarp -hardwood to northern podocarp -hardwood stream types.

Table 8.6 gives the distribution of each major species between streams in the leaf bag experiment and stream survey, the seasonal patterns seen in the leaf bag experiment and stream filters, and the temperature-growth response (minimum and optimum) where measured. The dominance of alpine streams by *V. elodeae* and *A. tetracladia* can be explained by their temperature-growth responses, both growing well at 1 C compared to many other aquatic hyphomycetes, whose minimum temperature for colony extension could be as high as 5 C. *F. penicillioides* was also relatively abundant in alpine streams but this species has a minimum temperature for growth of 5 C. The remainder of its distribution pattern, that is, greater abundance in

northern podocarp -hardwood streams than southern podocarp -hardwood and beech, and its seasonal pattern (summer maximum) fit in better with the temperature-growth response. The minimum temperature for growth of *L. ovalis* sp. ined. was slightly lower than for *L. cymbiformis* and *L. curvula*, but the latter species were much more widespread (all podocarp -hardwood and beech streams compared with northern podocarp -hardwood only). The difference in temperature -growth response matches the difference in seasonal periodicity however, *Lunulospora* spp. being more abundant in summer and *L. ovalis* sp. ined. in winter.

Thus a direct temperature effect on vegetative growth does not explain all the differences in seasonal periodicity or distribution among stream types. Nor can it explain why cold stream/winter species were less common at warmer temperatures, when both winter and summer species have similar temperature optima (which must be quite rarely attained, and certainly never exceeded, in the streams sampled).

Suberkropp (1984) explained the absence of warm site summer species in winter by their temperature -growth responses (no growth at or below 5 C). These warm site summer species could grow and maintain themselves at the colder sites (in summer) when artificially introduced, but did not occur there naturally. Suberkropp suggested that slightly higher temperatures were important to the initial establishment. Thus summer species began to establish dominance at warm sites as the temperature approached 20 C, then could compete effectively to maintain their dominance until the temperature dropped below the minimum for growth, when winter species established their dominance, continuing until the temperature again approached 20 C.

This mechanism may help explain seasonal patterns among species, but cannot explain the absence of northern podocarp -hardwood stream (warm site) species from more southerly streams in summer at least, for example *L. ovalis* sp. ined. was present in Hakarimata but not in Reid's Ck, despite equally high maximum summer temperatures. Possibly the minimum summer temperatures were more important in determining the occurrence of this species, the minimum temperatures of more southerly streams, including Reid's Ck, favouring cold tolerant species. In comparison, *Lunulospora* spp., despite having an even higher minimum temperature for growth, competed more successfully with species with lower minimum growth temperatures and was important in all but alpine streams, although more abundant in summer and podocarp -hardwood streams.

Thus the limiting effect of the temperature-growth response of a species is probably on the ability of the species to compete with others, as much as by directly inhibiting growth outside the limits for growth. The degree to which temperature is the major determining factor of distribution and seasonal patterns among aquatic hyphomycete species in the present study cannot therefore be determined easily. The best evidence that temperature is the controlling factor is that distribution among streams and seasonal patterns seem to coincide, and to show patterns which follow differences in temperature more closely than any other factor.

In conclusion, as a result of the work done by Bärlocher and others on pH and water hardness, Wood -Eggenschwiler and Bärlocher (1985) state that "within the same climatic zone, the water chemistry of the stream appears to be the overriding factor determining the composition of fungal communities". Gönczöl (1987) found a similar relationship between the aquatic hyphomycete flora

and water hardness longitudinally within a single stream. The streams in the present stream survey, although relatively few in number and sampled only once, suggest that smaller temperature changes than those occurring between climatic zones may have a controlling influence on aquatic hyphomycete community composition. Other factors such as pH and water hardness may also influence the fungal flora and its abundance, but were not as readily demonstrable from the present work, and so may play a more minor role in undisturbed New Zealand streams.

8.4.4 Potential Effect of Oxygen on Species Distribution and Abundance.

Aquatic hyphomycetes have acquired a reputation for intolerance of low oxygen conditions, largely by observation of their reduced abundance in low oxygen habitats. Thus Tan and Lim (1983) suggested the lack of aquatic hyphomycetes in polluted rivers and canals was due to low oxygen levels. Bärlocher (1982b) suggested that sediments on leaf surfaces affected aquatic hyphomycetes by decreasing the oxygen supply necessary for colonisation. Cummins et al (1980) claimed that litter processing in largely anaerobic habitats did not involve significant aquatic hyphomycete utilisation. However, little or no work has been done to directly determine the sensitivity to low oxygen conditions of different aquatic hyphomycetes. Nilsson (1964) found that although *A. tetracladia* grew more strongly in aerobic compared to anaerobic conditions there was no striking difference. He considered *A. tetracladia* to be an atypical species, being less common in fast flowing streams and more common in dystrophic habitats. Webster and Towfik (1972), in a study of the effect of aeration (turbulence) on

sporulation, stated: "Numerous investigators have shown that the rate of oxygen uptake by submerged cultures of microorganisms is independent of O_2 concentration over wide limits extending to quite low partial pressures. This finding was confirmed for submerged mycelium of *A. tetracladia* and *L. aquatica* using polarographic methods of O_2 concentration". According to Griffin (1972) most soil fungi showed little change in growth rate on agar until oxygen was lowered to about 4 %. Although there was considerable difference between species, nearly all were declining by 1 % oxygen. Emerson and Natvig (1981) state that "at least 20 common molds exhibit anaerobic growth".

In liquid culture the diffusion rate could limit respiration, and therefore growth, at somewhat higher concentrations. However it is clear from the present work (5.3.3) that *L. cymbiformis* was much more sensitive to decreasing oxygen concentrations than most soil fungi, respiration of submerged mycelium in sealed bottles decreasing markedly at a little below 10 % oxygen. This is surprising as low oxygen concentrations would occur more frequently in aquatic than terrestrial habitats. However, it does explain the apparent preference for clean, flowing water seen in most aquatic hyphomycetes.

In the present work the effect of low oxygen was not separated from that of high carbon dioxide, which would have increased in the sealed bottles as the oxygen was used up. Emerson and Natvig (1981) point out that increased carbon dioxide concentrations often accompany low oxygen concentration in field situations, so the high carbon dioxide levels in the present work were not a disadvantage in assessing the ability of an aquatic hyphomycete species to grow in low oxygen environments.

Where oxygen and carbon dioxide concentrations changed together it appears that the change in carbon dioxide rather than oxygen is the determining factor according to Griffin (1972). Carbon dioxide concentrations of up to 10 % cause little effect on the growth rate of many fungi in air (Griffin, 1972), but in submerged conditions may have a stimulatory or inhibitory effect depending on pH. Danielson and Davey (1973) found *Trichoderma* showed reduced growth in 2 to 10 % carbon dioxide on acid medium compared with increased growth on alkaline medium. No measurements of pH were made during the present work, so it is not clear whether carbon dioxide could have been having a stimulatory or an inhibitory effect. However, in view of the apparently high sensitivity to decreasing oxygen concentrations it seems more likely that increasing carbon dioxide levels were having an inhibitory effect, rather than stimulatory.

L. cymbiformis appeared to be more sensitive to low oxygen conditions than some other species in the nutrient enrichment experiment (5.3.3), notably *T. chaetocladium* and *C. aquatica*. In this experiment nutrient enrichment caused a greater decline in the levels of *L. cymbiformis* than the other species present. Thus one of the most abundant species in most of the New Zealand streams sampled appeared to be particularly sensitive to low oxygen conditions.

It might be predicted then, that aquatic hyphomycetes would decline noticeably in heavily polluted streams, or in other circumstances under which oxygen concentrations in the leaf substrate might be reduced, e.g. in silty streams, upon coating or burial of the leaves. A change in species composition could also be expected. This could be tested for in future streams surveys, using the present survey as a model of the undisturbed stream type.

APPENDIX 1: RIPARIAN FLORA OF REID'S CK.

The following species were identified from about or 200 m upstream of the experimental site in Reid's Ck (Tongariro National Park). Taxonomy after Allan (1961) and Moore and Edgar (1970).

Acaena spp.,

Aristotelia fruticosa,

A. serrata,

Astelia fragrans,

Blechnum capense,

B. colensoi,

B. fluviatile,

Brachycome sinclairii var. *sinclairii?*,

Carex sp.,

Carpodetus serratus

?Cassinia vauvilliersii,

Coprosma australis,

C. colensoi,

C. foetidissima,

C. parviflora,

C. tenuifolia,

Cordyline indivisa,

Dracophyllum sp.,

Epilobium sp.,

Fuschia excorticata,

Gaultheria antipoda,

Gleichenia cunninghamii,

Gnaphalium keriense,

Griselinia littoralis,

Helichrysum sp.,

Histiopteris incisor,

Leptospermum scoparium,

Libocedrus bidwillii,

Lycopodium fastigiatum,

L. volubile,

Mycelis muralis,

Myosotis forsteri,

Nothofagus menziesii,

N. solandri var. *cliffortioides*,

Olearia arborescens,

O. fragrans,

Ourisia ?macrophylla,

Phyllocladus alpinus,

Pittosporum colensoi,

Podocarpus totara,

Polystichum vestitum,

Pratia angulata,

Pseudopanax arboreus,

Pseudowintera colorata,

Rubus cissoides,

Schefflera digitata,

Uncinia sp.,

Viola sp.

APPENDIX 2: NOTES ON LEAF DECAY IN THE LEAF BAG EXPERIMENT.

Kamahi, first run, Hakarimata: Many of the leaves were waterlogged, slimy, and olive green after one month, and some were becoming soft and partially skeletonized, the epidermis often separating from the middle lamella during handling. One leaf showed a pattern of loss of interveinal tissue corrosponding to the mesh bag over it, as if worn away where not protected by the plastic. By 3 months (April 1983) almost all the leaves were partially skeletal and many almost entirely. The lamina was olive and the veins white to pale brown. After 5 months (June 1983) all leaves were skeletal, many reduced to mid rib and major veins only. In the next few months these remains reduced to nothing.

In the second run, most of the leaves were waterlogged, slimy, and discoloured (dull green, with some patches a reddish to dark brown) after one month also. However, by 3 months (August 1983) the leaves were dark brown to black, fairly stiff, small areas only chewed to approach a skeletal pattern. A small number of leaves showed a similar pattern of decay to the first run, olive green lamina being lost to reveal pale vein skeletons by 5 months (October 1983), but most were entire, dark and brittle, not slimy, but having a rather gritty texture as the outer layers of the leaf were chewed or worn away. Loss of leaf material occurred as patches of whole leaf, although a rough, dark brown to black skeletal framework of major veins frequently remained. The rate at which this occurred varied between leaves, but by 8 months (January 1984) only a few leaves were still recognisable. By 12 months (May 1984) they consisted of black fragments, mostly of entire lamina and chewed looking, but including some dark skeletal remains, mostly mid ribs

and petioles.

Fallen kamahi became similarly slimy and discoloured, all the leaves dark brown to black by 2 months (July 1983). There was a tendency, seen also in fresh kamahi in the second run, for numbers of petioles to dehisce and be lost at this early stage. At 4 months (September 1983) skeletonisation was proceeding well in some leaves but not in others, so that by 8 months most leaves were still entire, only a few reduced to fragments,mainly skeletal. By the end of the experiment (12 months) the leaves were fragmenting generally, but a few leaves still retained a discernable shape, although partly skeletonized and chewed looking.

In Reid's Ck, in the first run, kamahi became almost all waterlogged in the first month, dull green and slimy. Leaves retained their green colour largely, becoming worn away to pale skeletons at rates that varied greatly between individual leaves. By 5 months (May 1983) the leaves were dull olive brown and about half were skeletonized, the remainder partly entire or mostly entire. At 8 months (August 1983) only sparse, dirty white skeletons remained, which persisted in declining amounts till the end of the run.

In the second run, kamahi was slower to become waterlogged and discoloured, all being waterlogged, mostly dark brown, and lightly slimy only by 3 months (August 1983). But by 4 months about 2/3 of the leaves had areas of partial skeletonization as seen in Hakarimata. Parts of the leaves had been clearly chewed away, completely or leaving only an epidermis, in the following month. By 6 months (December 1983) the leaves were black, and some obviously brittle and fragmenting. In February (8 months) only fragments of entire and skeletal leaf material remained, softened slightly from

the rigid leathery feel of earlier. By the end of the experiment (11 months) only chewed looking leaf mid ribs and some major veins, some with fragments of lamina attached, remained.

Beech, first run, Hakarimata: After one month only half the leaves were waterlogged, in contrast to kamahi, the infiltrated areas dark brown, hard and rigid, not slimy. By 3 months (March 1983) leaves were dark brown to black, somewhat brittle, and over half of them chewed or damaged to some extent. Dark major veins with fragments of lamina attached and petioles persisted best as decay proceeded in the following months, but some fragments of entire leaf were found up to the last sample with any leaf material remaining: 8 months (August 1983).

In the second run beech leaves were 3/4 waterlogged and dull olive green after one month. In the following 2 months the leaves became dark brown and slightly brittle, but noticeably less slimy than kamahi. The first signs of chewing appeared after 4 months (September 1983), and almost all leaves were damaged to some extent by 5 months: chewed, eroded, partly skeletonized, or brittle and fragmenting. Only a few recognisable leaves remained by 7 months (December 1983), the rest present as small, largely entire, fragments. By March 1984 (10 months) no leaf material remained in any of the mesh bags.

In Reid's Ck in the first run, beech became waterlogged by the second month, turning dull olive or olive brown, but was not slimy to the touch like kamahi. By March 1983 (3 months) theleaves were stiff, dark brown to black, and showed the first signs of damage, possibly due to chewing. At 10 and 11 months (October and November 1983) only chewed fragments remained, the amount varying

considerably between bags. Some fragments still remained at the of the run (12 months).

In the second run beech was mostly waterlogged by 3 months (August 1983). All leaves were brown by 4 months. Chewing damage appeared by 6 months (November 1983), a few leaves reduced to fragments while the majority were entire and largely undamaged. All were dark brown. Partly chewed leaves, reduced to a skeletal framework, with or without epidermis, were seen in December, along with many partially fragmented leaves. By the last sample in the experiment (April 1984) fragments of entire leaf only remained, varying in amount between bags.

The fallen beech leaves (second run only) were largely waterlogged and discoloured to a dull mid brown or dull chestnut in the first month. Throughout the remaining period the leaves remained a mid to dark brown, slightly lighter than the fresh beech. By 7 months (December 1983) the leaves, unlike those of fresh beech, were undamaged and did not show increased brittleness. However, by the final sample (11 months, April 1984) about half the leaves were chewed to some degree, or appeared more brittle.

Hangehange, first run, Hakarimata: Although waterlogging of all the leaves of hangehange was not seen until the one month sample (January 1983), before this stage some leaves were slimy and decaying rapidly. Most of the leaves in January were dull olive, very slimy, and disintegrating, unable to be picked up individually. By 2 months there was a small amount of structureless material only remaining.

Kanono, first run, Hakarimata: All leaves of young kanono were waterlogged by the one month sample. They were a dull dark green

and slimy. Half the leaves were partly skeletonized and a number like hangehange, that is disintegrating together in an amorphous fashion. Some of this material was nearly blue -black and rather smelly in the February sample. Frequently, the middle lamella only was lost, leaving the epidermis and pale vein network intact. These were all that remained in the 4 and 8 month samples (April and August 1983), the vein network complete to the smallest veins, unlike kamahi. A small mass of fawn coloured vein fragments persisted to the 12 month sample (December 1983).

Most of the old kanono leaves were waterlogged by the one month sample, dark dull green, and the outermost leaves in the bag partly skeletonized. By 4 months most of the leaves skeletonized, with or without intact epidermis, and in some the finer veins were lost. By the 12 month sample (December 1983) the leaves consisted mainly of pieces of fawn coloured mid rib, somewhat eroded.

Kauri, first run, Hakarimata: Some waterlogging of young kauri commenced by 1 week, but not until 2 months were all leaves waterlogged. The leaves were dull olive green, darkening to black in parts, firm, but slimy to touch at this stage. By 12 months (December 1983) they had become mid brown and all leaves showed some chewing partly or entirely through the whole lamina. In the last sample of the experiment the leaf rims were intact but most leaves were reduced to a single epidermis or less within these. In several places where leaves were stuck together, however, the leaves were entire and pale brown, like a much earlier decay stage.

Old kauri leaves followed a similar pattern to young kauri, turning olive green to orange -brown by 2 months, then dark brown and chewed by 12 months.

The remaining four leaf types were sampled only twice during decay. At 6 months tawa leaves were largely olive to mid brown, with patches of skeletonized tissue or, more commonly, veins and epidermis. By 12 months the leaves consisted of petioles, with a few fragments of lamina and some midribs.

At 6 months the ponga fronds were entire, stiff, the lamina olive brown and stipe dull reddish brown to dark brown. By 12 months the lamina was a mottled light brown where still entire, but where damaged the lamina was almost all gone, chewed back to the petioles.

At 6 months nikau leaflets were dull green still, although considerable loss of areas of lamina or of middle lamella alone had occurred. Where vein networks remained these were reddish brown. Longitudinal splits, along the edge of the mid rib especially, occurred occasionally. By 12 months most of the interveinal tissue was lost, only fragments of epidermis remaining on finer longitudinal veins.

At 6 months rimu leaves were mid to dark brown, softened, some a bit ragged and chewed looking. A number of the tiny leaves were falling off the branchlets and being lost through the mesh of the bags. The branchlets varied greatly by 12 months. Some were dark brown to black, showing little leaf loss. Others had lost all the leaves and the stem cortex, leaving white wood exposed, some of which had itself become discoloured and partly eroded.

APPENDIX 3: INVERTEBRATE FAUNA.

The invertebrates seen in the leaf bag experiment belonged to the following orders. In Hakarimata: Annelida, Coleoptera (once), Diptera (chironomids and several others), Ephemeroptera (several species), Mollusca, Neuroptera, Plecoptera (several species), Trichoptera (several species); in Reid's Ck: Annelida, Decapoda (*Paranephrops*), Diptera (chironomids and others), Ephemeroptera (several species), Neuroptera, Plecoptera (several species), Trichoptera (several species).

Appendix 4: Aquatic hyphomycete mycelial content (total m stainable mycelium/g leaf material and percentage of this calculated for each species for which biomass/spore conversion factors exist) of first run (immersed in December) kamahi in the Hakarimata stream.

Species	Month									
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.
TOTAL (m/g)	341	643	8652	19271	5542	49189	42413			2398
A. acuminata	12.3	0	4.0	0	0	0	0			13.4
Alatospora spp.	0	0	14.2	6.3	1.4	1.6	0.3			0
A. filiformis	7.8	3.5	0	0	0	0	0			0
A. crassa+longiss.	0	0	4.0	57.1	78.9	57.1	49.3			40.0
C. aquatica	0	0	0.2	1.1	2.8	0.7	0.7			20.7
C.longibrachiata	0	38.1	55.6	27.0	9.0	41.6	57.3			26.6
F. penicillioides	1.3	2.6	0.1	0	0	0	0			0
Lunulospora spp.	58.1	23.0	2.1	0.1	0	0	0			0.1
L. ovalis	11.0	5.6	2.9	1.8	0	0.1	0.1			0
T. marchalianum	0	4.0	18.7	0	0	0	0			0
T. chaetocladium	15.6	22.6	7.9	6.1	8.3	0.2	0.9			0

Appendix 5: Aquatic hyphomycete mycelial content (total m stainable mycelium/g leaf material and percentage of this calculated for each species for which mycelium/spore conversion factors exist) of second run (immersed in May) kamahi in the Hakarimata stream.

Species	Month										
	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
TOTAL (m/g)		1279	20419	17458	1417	1449	271		168	44	32	
A. acuminata		0	0	0	1.5	8.2	46.4		8.0	0	0	
Alatospora spp.		28.5	45.5	66.7	38.5	55.6	41.3		84.1	80.0	87.0	
A. filiformis		0.3	0	4.0	0.2	0	0.2		0.3	0	0	
A. crassa+longiss.		1.2	0.9	0.8	4.4	6.8	0		0	0	0	
C. aquatica		1.1	2.2	1.9	4.1	4.6	4.1		1.3	1.6	2.2	
C.longibrachiata		8.8	0.2	0	0	0	0		4.0	0	0	
F. penicillioides		0	0	0	0	0	0		0	0	0	
Lunulospora spp.		12.2	6.0	2.6	9.1	1.9	0.7		0.1	3.5	0	
L. ovalis		20.1	26.7	9.4	7.5	0.9	5.8		0	0.6	0	
T. chaetocladium		24.2	15.7	9.7	33.9	21.4	1.0		1.7	9.6	8.7	

Appendix 6: Aquatic hyphomycete mycelial content (total m stainable mycelium/g leaf material and percentage of this calculated for each species for which mycelium/spore conversion factors exist) of first run (immersed in December) beech in the Hakarimata stream.

Species	Month								
	2 wk	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
TOTAL m/g	23	5345	8274	3274	3372	2081	893		35
A. acuminata	0	1.0	0	0	0	0	0		0
Alatospora spp.	0	1.9	3.0	61.4	34.7	9.4	9.4		0
A. filiformis	0	0.1	1.1	0.6	1.9	1.0	3.4		0
A. crassa+longiss.					1.3	2.4	3.9		0
C. aquatica	0	0.1	0.1	0.7	3.8	3.9	13.0		14.0
C.longibrachiata	0	4.1	4.8	2.1	2.7	0	0		0
F. penicillioides	20.9	0.1	0.3	0	0.1	0.1	0		0
Lunulospora spp.	0	46.1	25.9	4.2	1.0	2.4	0.6		1.2
L. ovalis	0	35.2	40.6	8.3	4.8	5.0	3.0		0
T. chaetocladium	0	10.9	23.6	21.4	48.8	75.5	64.6		60.0

Appendix 7: Aquatic hyphomycete mycelial content (total m stainable mycelium/g leaf material and percentage of this calculated for each species for which mycelium/spore conversion factors exist) of second run (immersed in May) beech in the Hakarimata stream.

Species	Month									
	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
TOTAL (m/g)	494	5653	17472	5669	1774	371	150	54	49	
A. acuminata	0	0	0.5	0	6.7	33.9	9.4	26.1	0	
Alatospora spp.	0	6.2	7.6	43.0	41.0	24.5	28.1	65.3	28.4	
A. filiformis	0	0.2	0.1	0	0	0	0	0	0	
A. crassa+longiss.	0	0	0	0.9	1.7	9.4	46.8	0	42.6	
C. aquatica	0	2.9	11.2	7.6	9.9	8.3	0.9	2.6	0	
C.longibrachiata	0	18.0	6.3	0	4.7	13.2	0	0	0	
F. penicillioides	0	0	0	0	0	0	0	0	0	
Lunulospora spp.	78.8	9.3	5.3	3.8	1.8	0.5	8.8	0	0	
L. ovalis	13.5	36.0	46.0	24.5	13.8	0.9	0.4	1.0	0	
T. chaetocladium	6.8	26.9	20.6	19.0	20.0	6.8	5.6	2.6	.4	

Appendix 8: Aquatic hyphomycete mycelial content (total m stainable mycelium/g leaf material and percentage of this calculated for each species for which mycelium/spore conversion factors exist) of first run (immersed in December) kamahi in Reid's Ck.

Species	Month										
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
TOTAL (m/g)	103	1052	9660		12110	34860	23177	540	942	2098	?	?
A. acuminata	0	0	4.5		4.8	11.1	25.6	24.6	51.3	15.0		
Alatospora spp.	0	0	5.6		4.7	0	2.2	0	0	18.7		
A. filiformis	59.8	12.4	5.0		0.8	0.3	0.3	0	0	1.6		
A. crassa+longiss.	0	0	0		0	0.5	3.1	0	0	3.8		
A. tetracladia	0	0	0.6		0.5	0.2	0.4	0	0.4	1.3		
C. aquatica	0	0	0.4		3.5	5.9	13.6	2.5	2.9	13.7		
C.longibrachiata	13.6	51.2	56.1		71.5	76.3	47.3	13.0	0	33.4		
Filosporella sp.	0	0	0.3		0.5	0.1	0.2	0.6	0	0.7		
L. aquatica	0	0	0		0	0.3	0	0	0	0		
Lunulospora spp.	0.9	0.5	3.4		1.3	0.3	0.7	0.9	8.3	1.5		
T. elegans	2.7	0.4	1.3		0	0.2	0	2.6	0	0		
T. chaetocladium	0	33.5	21.8		7.6	2.7	1.7	53.2	25.4	7.8		

Appendix 9: Aquatic hyphomycete mycelial content (total m stainable mycelium/g leaf material and percentage of this calculated for each species for which mycelium/spore conversion factors exist) of second run (immersed in May) kamahi in Reid's Ck.

Species	Month										
	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
TOTAL (m/g)	27	1609	8582	3970	4933	3580	6266	2175	2088	1870	496
A. acuminata	7.7	7.4	0	0.5	3.8	10.0	4.1	8.4	42.2	12.4	50.8
Alatospora spp.	0	0	0.7	1.1	0.9	4.3	6.6	2.3	14.1	0	14.1
A. filiformis	0.5	17.9	17.6	15.0	23.4	6.8	10.8	13.8	1.6	1.6	1.6
A. crassa+longiss.	0	0	0	0	0	3.3	0	2.3	0	0	0
A. tetracladia	0	2.4	0.5	2.4	5.9	2.9	1.4	0.3	1.4	0	1.7
C. aquatica	2.3	0	0	0.8	0.8	6.4	8.2	7.7	6.5	3.3	4.7
C.longibrachiata	0	16.5	0.6	0	0	9.4	21.9	3.2	10.4	12.4	0
Filosporella sp.	0	0	0	0	0	0	0	0	0	0.3	0
F. eccentrica	0	0.3	1.2	4.0	4.6	10.2	3.1	1.2	1.5	0.2	1.3
L. aquatica	80.0	1.4	0.6	0.6	0.5	0.7	0.3	0.6	0.1	0	0
Lunulospora spp.	0.2	0.2	0.2	1.8	0.9	1.5	1.2	3.3	1.1	0.3	0.3
T. elegans	4.6	52.2	63.5	56.7	38.5	10.3	15.1	3.0	1.0	0	0
T. chaetocladium	3.1	0.8	10.9	15.1	25.3	7.2	7.7	7.7	3.2	2.4	6.5

Appendix 10: Aquatic hyphomycete mycelial content (total m stainable mycelium/g leaf material and percentage of this calculated for each species for which mycelium/spore conversion factors exist) of first run (immersed in December) beech in Reid's Ck.

Species	Month											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
TOTAL (m/g)	20	963	3513	3004	2871	4955	4572	837	2052	4754	1023	198
A. acuminata	0	0	11.6	7.9	27.6	25.0	33.1	40.1	23.1	13.4	52.7	38.9
Alatospora spp.	0	5.8	18.3	7.2	8.0	13.1	11.9	30.9	1.5	29.9	15.7	0
A. filiformis	6.7	5.3	18.6	18.6	10.9	5.6	1.5	1.6	6.2	3.0	2.2	2.5
A. crassa+longiss.	0	0	0	0	0	0	1.4	0	0	0	0	0
A. tetracladia	0	1.0	1.6	1.5	1.0	0.3	0.3	0	0.3	0.3	0.2	2.0
C. aquatica	0	0.4	9.7	2.7	1.7	26.5	27.3	16.7	18.4	29.5	5.3	0
C.longibrachiata	71.2	74.1	12.4	31.5	14.9	0	0	0	1.5	1.2	0	0
Filosporella sp.	0	0	0	0	0	0.1	0	0.1	0	0	0.1	0
Lunulospora spp.	0	0.1	12.4	9.3	15.0	18.5	8.8	7.0	39.2	16.6	15.1	8.4
T. elegans	21.4	9.7	6.8	6.9	1.5	0.6	0.2	0.5	0	0	0.4	0
T. chaetocladium	0	2.5	2.6	4.9	8.9	18.5	0.8	0	0.7	1.8	1.5	7.8

Appendix 11: Aquatic hyphomycete mycelial content (total m stainable mycelium/g leaf material and percentage of this calculated for each species for which mycelium/spore conversion factors exist) of second run (immersed in May) beech in Reid's Ck.

Species	Month										
	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
TOTAL (m/g)	35	1028	6480	10948	4574	2098	4630	3218	2208	4347	5316
A. acuminata	0	0	0.5	3.3	12.4	28.4	20.0	7.6	38.0	32.0	53.6
Alatospora spp.	0	0	0.5	0.8	4.0	5.3	7.0	39.4	13.6	37.8	19.7
A. filiformis	0.4	0.9	1.5	3.3	6.4	3.5	5.1	3.6	1.5	0.3	1.0
A. crassa+longiss.	0	0	0	0	0	0	0	0	0	0	0
A. tetracladia	0	0.1	0.5	0.7	0.7	1.0	0.4	0.8	0.4	0.5	0.7
C. aquatica	0.1	0.1	0.5	0.5	3.7	0.8	27.1	9.8	1.2	2.9	0.9
C.longibrachiata	0	79.7	75.5	59.7	30.1	30.0	14.8	11.5	6.0	3.9	0.8
Filosporella sp.	0	0	0	0	0	0	0	0	0	0.3	0.1
F. eccentrica	0	0	0.1	0.4	0.3	1.0	0	0.1	0.1	0.1	0.2
Lunulospora spp.	0	0	0.3	3.9	7.6	2.2	4.0	2.2	3.2	1.0	1.0
T. elegans	0	15.8	16.8	19.2	11.6	11.6	5.2	2.9	8.5	3.9	4.1
T. chaetocladium	0	0	1.3	6.4	11.1	3.5	11.2	14.6	1.6	6.2	2.1

Appendix 12: Spore production by the major, successional, species recorded from hangehange, young and old kanono, and young and old kauri compared with that from first run kamahi in the Hakarimata stream. All leaf types were introduced initially in December. Species are grouped into early, mid, and late decay species (approximately, *C. longibrachiata* varying between leaf types). The mean spore production is given first, followed by the standard deviation. Percent weight loss for each leaf type is given in parallel.

Species	Time to sampling														
	1 wk	2 wk	1 m	2m	3m	4m	5m	6m	7m	8m	9m	10m	11m	12m	18m
KAMAHI															
% weight loss:	13	15	40	50	55	58	96	100	100			100			
F. penicillioides		11.1	29.4	107.5	47.0		0.3								
		/19.1	/32.4	174.2	/46.5		/0.6								
A. filiformis			0.6	5.1											
			/1.1	/4.6											
Lunulospora spp.			141.5	105.6	128.8	17.8	0.9	9.4	1.6			2.3			
			/97.7	/49.4	/33.2	/14.6	/1.6	/11.0	/2.7			/-			
L. ovalis			26.9	25.7	179.8	253.3	1.3	24.3	25.9						
			/41.7	/31.3	/81.2	/53.0	/2.3	/6.5	/20.0						
T. marchalianum				7.2	454.2										
				/12.6	/264.8										
T. chaetocladium			3.8	10.4	48.6	83.4	32.7	5.5	26.7						
			/1.8	/2.3	/18.6	/22.3	/31.5	/1.1	/37.8						
Alatospora spp.					17.5	17.3	1.0	11.0	1.6						
					/6.7	/17.7	/1.7	/10.9	/2.7						
A. acuminata	0.5		0.6		5.0										
	/0.9		/1.1		/2.9										
A. crassa+longiss.					5.2	157.1	62.5	401.1	250.5			13.7			
					/1.7	/108.4	/16.0	/35.3	/68.6			/-			
C. aquatica					2.2	29.5	21.9	48.0	41.6			70.7			
					/3.9	/11.7	/16.1	/22.3	/26.1			/-			
C. longibrachiata				3.5	68.7	75.2	7.1	292.5	352.7			9.1			
				/0.8	/36.4	/51.9	/9.5	/39.8	/197.5			/-			

Appendix 12 cont.

	1 wk	2 wk	1 m	2 m	3 m	4 m	5 m	6 m	7 m	8 m	9 m	10 m	11 m	12 m	18 m
HANGEHANGE															
% weight loss:	14	33	54												
F. penicillioides	0.9														
	/1.0														
A. filiformis	0.2	0	15.3												
	/0.3		24.4												
Lunulospora spp.	11.5	1.2													
	/2.9	/0.8													
L. ovalis	11.2	3.3	9.4												
	/1.2	/5.0	/1.5												
T. marchalianum															
T. chaetocladium	1.2														
	/2.0														
Alatospora spp.															
A. acuminata															
A. crassa+longiss.															
C. aquatica															
C. longibrachiata															

YOUNG KANONO				
% weight loss:	0	51	46	89
F. penicillioides		0.2	7.6	17.0
		/0.4	/9.1	/26.2
A. filiformis				
Lunulospora spp.		2.4	11.3	0.7
		/2.7	/11.6	1.2
L. ovalis		0.3	3.9	1.3

Appendix 12 cont.

	1 wk	2 wk	1 m	2 m	3 m	4 m	5 m	6 m	7 m	8 m	9 m	10 m	11 m	12 m	18 m
			/0.6	/6.7		/1.6									
T. marchalianum				3.9											
				/6.7											
T. chaetocladium			0.3	5.8		0.7									
			/0.6	/10.1		/1.2									
Alatospora spp.						0.2									
						/0.3									
A. acuminata				1.7											
				/3.0											
A. crassa+longiss.						0.2									
						/0.4									
C. aquatica															
C. longibrachiata			0.2	7.4		0.7									
			/0.4	/7.4		/1.2									
OLD KANONO															
% weight loss:		10	27	70		92				85				100	
F. penicillioides			1.5			1.4									
			/1.3			/2.4									
A. filiformis															
Lunulospora spp.		0.1	92.3	7.9		6.8								0.3	
		/0.2	/65.7	/6.9		/4.3								/-	
L. ovalis			96.5	21.7		75.7				3.8					
			/91.5	/35.8		/64.7				/6.5					
T. marchalianum			27.7												
			/45.9												
T. chaetocladium			8.8	0.9		8.5								0.3	
			/8.4	/1.5		/11.1								/-	
Alatospora spp.						1.5								6.0	
						/2.5								/-	

Appendix 12 cont.

	1 wk	2 wk	1 m	2 m	3 m	4 m	5 m	6 m	7 m	8 m	9 m	10 m	11 m	12 m	18 m
A. acuminata										0.5					
										/0.9					
A. crassa+longiss.						22.7				87.0				3.7	
						/24.7				/117.3				/-	
C. aquatica		0.1				13.7				105.4				20.1	
		/0.1				/12.8				/161.5				/-	
C. longibrachiata						28.6				54.8				3.8	
						/19.5				/21.2				/-	
YOUNG KAURI															
% weight loss:			10	27		18				22				-	78
F. penicillioides				5.8		52.0				19.1				6.0	0.5
				/7.5		/61.6				/10.5				/7.8	/0.8
A. filiformis															
Lunulospora spp.			0.1	1.9		76.9				18.1				0.5	
			/0.1	/1.4		/78.8				/9.2				/0.9	
L. ovalis				2.0		249.6				894.0				200.9	435.0
				/2.0		/155.1				/246.7				/65.2	/284.4
T. marchalianum															
T. chaetocladium			0			48.9				28.8				15.5	31.3
			/0			/13.0				/9.9				/8.9	/24.5
Alatospora spp.						0.4				7.2				1.0	1.0
						/0.6				/3.7				/0.9	
A. acuminata										0.3				0.3	0.5
										/0.6				/0.6	/0.8
A. crassa+longiss.										0.5					0.9
										/0.5					/0.8
C. aquatica										1.1				0.3	
										/1.0				0.6	
C. longibrachiata			0			9.3				35.5				17.1	0.5
			/0			/5.0				/12.4				/11.1	/0.8

Appendix 12 cont.

	1 wk · 2 wk	1 m	2 m	3 m	4 m	5 m	6 m	7 m	8 m	9 m	10 m	11 m	12 m	18 m
OLD KAURI														
% weight loss:		9	26		27				30				69	90
F. penicillioides		0	63.6		527.2				371.7				29.0	2.6
		/0	/57.8		/300.0				/168.0				/-	/0.9
A. filiformis														
Lunulospora spp.			32.8		27.2				328.3				0.3	0.4
			/21.0		/24.0				/125.9				/-	/0.7
L. ovalis			14.7		429.3				994.5				48.0	7.1
			/3.2		/337.2				/479.7				/-	/3.3
T. marchalianum														
T. chaetocladium			1.2		8.3				68.2				17.5	28.8
			/0.5		/3.6				/15.0				/-	/6.0
Alatospora spp.									0.5				0.6	0.2
A. acuminata														
A. crassa+longiss.					0.4				0.2					
					/0.7				/0.3					
C. aquatica									0.3				2.1	
									/0.2				/-	
C. longibrachiata													2.1	0.4
													//-	0.7

Appendix 13: Spore production by the major, successional, species recorded from fallen kamahi, tawa, nikau, ponga, and rimu, compared with that from second run kamahi in the Hakarimata stream. All leaf types were introduced initially in May. Species are grouped into early, mid, and late decay species (approximately, *C. longibrachiata* varying between leaf types). The mean spore production is given first, followed by the standard deviation. Percent weight loss for each leaf type is given in parallel.

Species	Time to sampling													
	1 wk	2 wk	1 m	2m	3m	4m	5m	6m	7m	8m	9m	10m	11m	12m
KAMAHI														
% weight loss:	5	10	22	41	42	54	70	73	80	92	93	89	94	100
F. penicillioides			0.7	0.4	2.6									
			/0.6	/0.6	1.0									
A. filiformis				0.7	49.0	153.7	0.5		0.1	0.3	0.1			
				/0.8	/18.7	/50.0	/0.8		/0.2	0.2	0.1			
Lunulospora spp.			4.3	111.9	872.1	318.9	91.7	20.1	1.4	0.9	0.1	1.1		
			/4.5	/53.9	/858.6	/201.0	/13.1	/9.1	/1.0	/1.2	/0.1	/0.4		
L. ovalis			3.5	183.3	3880.8	1173.5	76.4	9.2	11.3	1.3	0	0.2		
			/6.1	/46.1	/114.8	/412.4	/45.9	/13.5	/10.6	/1.6	/0	0.2		
T. marchalianum				1.0	10.1	9.0				0	0	0.1	0.1	
				/1.7	/12.6	/8.9				/0	/0	/0.1	/0.1	
T. chaetocladium			1.0	22.1	228.0	121.4	34.3	22.2	0.2	1.9	0.2	0.3	0.2	0.1
			/1.7	/5.5	/28.4	/8.2	/20.7	/8.5	/0.3	/1.9	/0.1	/0	/0.3	/0.1
Alatospora spp.				5.2	132.6	166.3	7.8	11.5	1.6	1.4	2.1	0.5	0.4	0.4
				/7.6	/9.8	/33.5	/4.0	/2.6	/0.6	/0.2	/2.4	/0.1	/0.2	/0.4
A. acuminata							0.3	1.7	1.8	0.5	0.2			
							/0.6	/0.4	/0.7	/0.2	/0.1			
A. crassa+longiss.			0.6	0.2	2.5	1.8	0.9	1.4						
			/1.1	/0.3	/2.8	/1.6	/0.9	/0.7						
C. aquatica				1.8	63.8	46.0	8.3	9.5	1.6	0.7	0.3	0.1	0.1	
				/1.6	/11.8	/16.4	/9.8	/7.5	/0.7	/1.0	/0.5	/0.1	/0.1	
C. longibrachiata				1.6	0.7	9.6					0.1			
				/2.7	/1.2	/7.2					/0			

Appendix 13 cont.

Species	Time to sampling												
	1 wk	2 wk	1 m	2m	3m	4m	5m	6m	7m	8m	9m	10m	11m	12m
FALLEN KAMAHI														
% weight loss:	6	12	14	13		53				61				91
F. penicillioides			8.1	8.7		0.4								
			/6.1	/4.1		/0.7								
A. filiformis			6.9	7.3		0.8								
			/11.9	/2.8		/1.3								
Lunulospora spp.			108.4	1286.7		249.0				1.1				
			/72.5	/406.2		/96.1				/-				
L. ovalis			428.4	2517.7		629.1				0.5				0.1
			/284.2	/1207.7		/566.1				/-				/0.1
T. marchalianum														
T. chaetocladium			6.1	430.0		10.0				0				
			/5.6	/9.5		/4.4				/0				
Alatospora spp.			0.4	4.8		18.6				0.4				0.2
			/0.7	/3.0		/7.4				/-				/0.3
A. acuminata						1.6				0.1				0.1
						/1.8				/-				/0.1
A. crassa+longiss.														
C. aquatica				2.4		3.3				0				
				/2.1		/0.8				/0				
C. longibrachiata														

Appendix 13 cont.

Species	Time to sampling												
	1 wk	2 wk	1 m	2m	3m	4m	5m	6m	7m	8m	9m	10m	11m	12m
TAWA				NIKAU				PONGA				RIMU		
	6m	12m			6m	12m			6m	12m			6m	12m
% weight loss:	37	94			38	81			25	59			40	84
F. penicillioides	1.3	0			0	0.1							28.8	
	/1.3	/0			/0	/0.1							/8.4	
A. filiformis	0.4	0											0.7	0
	/0.7	/0											/0	/0
Lunulospora spp.	64.7				0.3				0.4				85.9	0
	/15.6				/0.2				/0.3				/41.8	/0
L. ovalis	15.2	0.1			0	0.1			0.3	0.3			10.9	0
	/7.9	/-			/0	/0.1			/0.4	/0.4			/8.6	/0
T. marchalianum	84.7								0	0			8.8	0
	/98.7								/0	/0			/4.9	/0
T. chaetocladium	23.3				1.2	0.1			0.5	0.1			28.2	0
	/9.1				/0.3	/0			/-	/0.2			/6.8	/0
Alatospora spp.	123.0	0.7			0.5	0.1			5.0	2.6			109.3	0.3
	/41.1	/-			/0.1	/0			/1.3	/1.8			/65.3	/0.2
A. acuminata		0							18.7	33.3			3.0	
		/0							/10.8	/9.1			/2.0	
A. crassa+longiss.	0.7				0.6	0.3			0				0.2	
	/0.7				/0.1	/0.3			/0				/0.4	
C. aquatica	134.1	0			0.2	0.3			4.2	2.0			0.9	
	/11.9	/0			/0.1	/0.4			/0.8	/0.7			/0.4	
C. longibrachiata	0.2													0.1
	/0.4													/0.2

Appendix 14: Spore production by the major, successional, species recorded from fallen beech compared with that from second run beech in Reid's Ck. All leaf types were introduced initially in May. Species are grouped into early, mid, and late decay species (approximately, *C. longibrachiata* varying between leaf types). The mean spore production is given first, followed by the standard deviation. Percent weight loss for each leaf type is given in parallel.

Species	Time to sampling											
	1 m	2m	3m	4m	5m	6m	7m	8m	9m	10m	11m	12m
BEECH												
% weight loss:	0	4	12	17		44	32	92	68	100	89	
H. lugdunensis		1.2	0.5	1.2								
		/1.6	/0.4	/0.1								
A. filiformis	0	2.0	21.4	80.9	66.1	16.5	53.2	26.1	7.6	2.8	12.5	
	/0	/1.5	/6.3	/8.9	/-	/4.7	/24.4	/9.0	/3.0	/2.5	/8.0	
A. tetracladia		0.2	8.7	22.7	9.5	6.1	4.7	7.3	2.4	5.7	11.0	
		/0.3	/5.7	/1.0	/-	/1.4	/1.8	/5.1	/1.5	/4.1	/1.7	
Lunulospora spp.			15.2	302.3	247.2	33.5	133.1	50.1	50.4	31.3	37.7	
			/6.8	/113.9	/-	/9.3	/63.0	/37.3	/17.4	/2.8	/6.6	
T. elegans		11.6	77.6	150.4	37.8	17.4	17.1	6.6	13.4	12.2	15.5	
		/1.5	/13.1	/57.0	/-	/1.0	/5.6	/4.5	/5.0	/10.9	/4.4	
T. chaetocladium			6.1	50.1	36.3	5.2	37.0	33.5	2.5	19.4	7.9	
			/4.3	/13.9	/-	/3.0	/17.4	/13.6	/0.7	/8.8	/2.7	
Unident. s/r 1(2)			6.8	43.7	60.5	37.5	68.4	21.8	15.4	29.9	28.2	
			/3.7	/9.2	/-	/7.7	/21.2	/5.2	/3.3	/31.5	/20.1	
A. acuminata			0.5	5.1	8.1	8.5	13.2	3.5	12.0	19.9	40.7	
			/0.8	/0.2	/-	/4.0	/7.5	/2.1	/3.3	/3.6	/10.1	
C. aquatica	0	0.2	5.0	7.9	24.1	2.4	179.3	45.0	3.8	17.8	7.2	
	/0	/0.3	/2.5	/7.5	/-	/1.6	/112.5	/9.7	/2.5	/20.4	/1.7	
C. longibrachiata		11.7	69.9	93.4	19.7	9.0	9.8	5.3	1.9	2.4	0.6	
		/2.6	/23.5	/59.5	/-	/1.29	/2.1	/3.5	/1.1	/1.2	/0.5	

Appendix 14 cont.

Species	Time to sampling											
	1 m	2m	3m	4m	5m	6m	7m	8m	9m	10m	11m	12m
FALLEN BEECH												
% weight loss:	6	4		13			32				58	
H. lugdunensis												
A. filiformis	3.7	1.1		15.4			1.6				1.8	
	/1.5	/0.5		/10.2			/0.5				/1.2	
A. tetracladia	0.4	0.5		3.3							0.2	
	/0.3	/0.9		/3.5							/0.2	
Lunulospora spp.	0.2			23.6			34.4				8.6	
	/0.4			/22.5			/11.0				/3.6	
T. elegans	6.8	2.4		6.6			0.5				0.5	
	/1.6	/0.7		/6.5			/0.8				/0.5	
T. chaetocladium				7.2			1.8				0.5	
				/4.8			/0.9				/1.1	
Unident. s/r 1(2)		0.5		2.9			2.6				2.8	
		/0.9		/2.8			/2.3				/1.1	
A. acuminata	0.2	2.3		2.9			10.9				10.7	
	/0.3	/1.9		/2.8			/3.6				/1.9	
C. aquatica				0.9			18.3				0.3	
				/0.4			/1.6				/0.5	
C. longibrachiata		0.8		23.3			8.1				0.1	
		/0.7		/4.3			/2.4				/0.2	

Appendix 15: Factors measured from streams sampled in the stream survey. The map references are given first, followed by the sample date, then latitude, altitude, size or discharge (where measured), 5 Day Biological Oxygen Demand (where measured), riparian vegetation, pH (where measured), water temperature (where measured). Water samples were filtered from each stream, and leaf samples collected from many. The number of aquatic hyphomycete species observed in each sampling method for each stream is given following the above physical parameters, followed by the total spores/l (water samples) or spores/g (leaf samples) measured, then the percentage of this total derived from each species (water sample/leaf sample).

	Stream								
	Olivine R. S. Westland	Rock Burn S. Westland	Unnamed str S. Westland	McTavish Ck S. Westland	Copland trib. Westland	Leslie R. NW Nelson	Cobb R. NW Nelson	Hannah Ck NW Nelson	Roaring Lion NW Nelson
Map Ref.:	S113/186363	S113/188139	S105/255605	S106/411571	S78/518478	S13/934351	S13/983522	S13/982522	S12/855397
Date:	31/1/84	4/2/84	24/1/84	21/1/84	28/1/85	7/1/84	8/1/84	9/1/84	10/1/84
Latitude:	44° 29'	44° 40'	44° 17'	44° 19'	43° 36'	41° 16'	41° 05'	41° 05'	41° 18'
Altitude (m):	550	700	1000	450	120	270	900	900	240
Size/Discharge (m^3/sec):	large	large	small	medium	v. small	large	large	small	large
B.O.D.$_5$:									
Vegetation:	Mtn beech	Mtn beech	alpine	Mtn beech + mixed rainforest	podocarp-hardwood	Red beech + p-hardwood	Beech	Beech	Beech + p-hardwood
pH:									
Temperature:									
No. spp. (water):	17	22+	9+	19	17+	15+	19+	16	16+
No. spp. (leaves):		20			15			18	
Total spores/l:	236	192	70	241	1188	9328	392	1089	2533
Total spores/g:		23231			137823			51296	
A. acuminata	4	4 /35	6	1	7 /<1	2	8	17 /23	1
Alatospora sp.	6	2 /2	9		3	7	4	3 /2	4
A. crassa+longiss.		2+2 /0+1		2+2	2+1		1+0	/0+<1	
A. filiformis									
A. tetracladia		0.5 /1		2	5		4	5 /<1	1
C. sphagnorum		0.5							

Appendix 15: cont.

	Olivine R.	Rock Burn		Unnamed str	McTavish Ck	Copland trib.		Leslie R.	Cobb R.	Hannah Ck		Roaring Lion
C. filicladia												
Ceratosporium sp.												
C. aquatica	1	4	/<1		12	1		>1	1			1
C. longibrachiata	4	2	/<1	7	8	6	/1	2	5	3	/1	6
											/<1	
Culic. aquatica												
D. aquatica		0.5										
E. microaquatica												
Filosporella sp.	4		/<1		5	4	/<1	>1		1		>1
F. penicillioides	3	17		34	24	1			1	3		1
F. eccentrica			/<1				/1				/<1	
Gyoerffyella sp.												
H. stellatacula												
H. campanulata				1								
L. uniinflata	0.5				1							
H. lugdunensis		2	/8						1	1	/<1	
L. aquatica		2			7	5	/36	<1	2	2	/<1	2
L. ovalis					14?			<1				
Lunulospora spp.	12	2	/15		3	41	/11	42	38	37	/46	46
Mycocentrospora sp	2	6			7							
Pleuropedium sp.					1							
T. gracilis			/3	1					4		/6	
T. elegans	1	2	/17		1	8	/40	3	5	2	/11	15
T. marchalianum						1		<1				<1
T. setigerum							/<1					
Tetracladium sp.												
Tetracladium sp	2											
T. aquatica												
T. chaetocladium	2		0.5	/<1		6	/1	2	1			4
T. curvatum	0.5											
T. splendens												
Tricladium sp.												
T. acuminatus												
T. myrti				/1								
Triposporina sp												
V. elodeae												

Appendix 15: cont.

	Olivine R.	Rock Burn		Unnamed str	McTavish Ck	Copland trib.		Leslie R.	Cobb R.	Hannah Ck		Roaring Lion
Unident. sp. 1							/3					
Unident. sp. 2		4								5		
Unident. s/r 1(2)								39	8	1	/5	16
Unident. s/r 1(3)	5	2	/15	13	3	4	/<1	<1	3	15	/5	1
Unident. s/r 2	0.5	4			1				1			
Unident. s/r 4												
Unident. s/r 10	16	37	/<1			4	/<1	<1	3	2		
Unident. s/r 12			/2	4							/<1	
Unident. s/r 14	39	5	/3	17	1		/<1					
Unident. s/r 15												
Unident. s/r 18								<1		1	/2	3
Unident. s/r 19												
Unident. s/r 21												
Unident. s/r 22				1						2		
unident. tetrarad				1	2	1		<1				
unident. sigmoid	1	0.5		1					10			1

Appendix 15 cont. (further sites).

	Stream								
	Tauranga R. (Ureweras)	Tataweka str (Ureweras)	Mauri str. (Ureweras)	Onepn str. (Ureweras)	Onepn trib. (Ureweras)	Unnamed str (Rotopunamu)	Mangatepopo (T.N.P)	Wairere str (T.N.P)	Unnamed str. (T.N.P.)
Map Ref.:	N87/562622	N87/666666	N87/494699	N87/497808	N87/497808	N112/218978	N112/092823	N112/061756	N112/058753
Date:	29/12/84	31/12/84	31/12/84	1/1/85	1/1/85	22/12/84	20/12/84	21/12/84	21/12/84
Latitude:	38° 28'	38° 26'	38° 25'	38° 19'	38° 19'	39° 01'	39° 09'	39° 12'	39° 12'
Altitude (m):	380	380	380	230	230	350	1200	1200	1200
Size/Discharge (m^3/sec):	medium	med-small	small	med-small	small	0.228m^3/sec	0.102m^3/sec	0.471m^3/sec	0.005m^3/sec
B.O.D.$_5$:				2.56		0.50	0.50	0.83	0.83
Vegetation:	Mtn beech + mixed hardwood	Mtn beech + mixed hardwood	Mtn beech + mixed hardwood	mixed hardwood	mixed hardwood	podocarp-hardwood	alpine	alpine	alpine
pH:						7.6	6.1	6.6	6.4
Temperature (°C):						12.5	14.0	9.75	17.5

Appendix 15 cont.

	Tauranga R.	Tataweka str	Mauri str.	Onepn str.		Onepn trib.		Unnamed str		Mangatepopo		Wairere str		Unnamed str.	
No. spp. (water):	21+	19	19	17+		15+		21+		6		3		3	
No. spp. (leaves):				16		11		14		8		12		6+	
Total spores/l:	2069	626	638	1352		1181		3770		56		6		6	
Total spores/g:				213307		960685		410802		34737		56489		3306	
A. acuminata		8	1	1	/1	1	/<1	5	/<1	20	/1	17	/52	33	/78
Alatospora sp.	4	10	9	8	/3	4	/1	3	/<1				/4		
A. crassa+longiss.	2+1	<1	2	1+0	/<1			<1+0							
A. filiformis	4							19	/8						
A. tetracladia	3	1	1	<1				2	/1	20	/79		/9		/15
C. sphagnorum															
C. filicladia															
Ceratosporium sp.	3							11	/<1						
C. aquatica		1	4	1	/<1	1		1							
C. longibrachiata		3	5	4	/<1	2		1	/<1				/11		
Culic. aquatica															
D. aquatica	<1														
E. microaquatica															
Filosporella sp.		3	1												
F. penicillioides	6	9	3	7	/5	6	/1	4					/2		
F. eccentrica			1						/<1	6	/<1	67	/5		
Gyoerffyella sp.						1									
H. stellatacula		3		<1											
H. campanulata															
L. uniinflata					/<1										
H. lugdunensis				<1	/1	1	/<1		/<1				/2		/1
L. aquatica	1		1					<1?							
L. ovalis	2?	2?	2?								/<1		/2		/1
Lunulospora spp.	42	23	39	29	/64	51	/80	24	/87						
Mycocentrospora sp															
Pleuropedium sp.															
T. gracilis										20	/1		/2		/1
T. elegans	2	3	16	5	/12	11	/14								
T. marchalianum	2	1	3	1	/<1	3	/<1								

Appendix 15 cont.

	Tauranga R.	Tataweka str	Mauri str.	Onepn str.		Onepn trib.		Unnamed str	Mangatepopo	Wairere str	Unnamed str.	
T. setigerum			1									
Tetracladium sp.												
Tetracladium sp 2								1				
T. aquatica												
T. chaetocladium	1	1	1	1	/<1	1	/1	1	/<1	/1		
T. splendens											17	
Tricladium sp.												
T. acuminatus		2						2			/<1	
T. myrti	1			<1		1		1	/<1			
Triposporina sp												
V. elodeae										/18	/6	
Unident. sp. 1				29	/11	8	/1					
Unident. sp. 2				4	/1	9						
Unident. s/r 1(2)	12	17						10	/1			
Unident. s/r 1(3)	1	4	3					3	/<1		/8	/2
Unident. s/r 2	3	1	3			1		1				
Unident. s/r 4	2									/<1		
Unident. s/r 10		9	6	9	/1	10	/<1	<1				
Unident. s/r 12							/<1					
Unident. s/r 14								1				
Unident. s/r 15	6							3				
Unident. s/r 18												
Unident. s/r 19												
Unident. s/r 21												
Unident. s/r 22	<1							<1				
unident. tetrarad									/<1			
unident. sigmoids	9			<1								/3

Appendix 15 cont. (further sites).

	Stream									
	Waipakahi trib.	Hapuawhenua	Reid's Ck	Waihora str	Rangitukia	Rangitukia 2	Hakarimata	Swanson str	Cascades str	Nihotupu
	Kaimaniwas	T.N.P.	T.N.P.	Pureora	Pirongia	Pirongia	Waikato	Auckland	Auckland	Auckland
Map Ref.:	N112/282718	N121/908582	N121/975565	N93/154433	N65/632257	N65/634256	N56/630602	N41/082568	N41/057557	N41/064506
Date:	22/12/84	21/12/84	21/12/84	22/12/84	16/2/85	16/2/85	16/2/85	17/3/85	17/3/85	17/3/85
Latitude:	39° 14'	39° 23'	39° 22'	38° 39'	37° 58'	37° 58'	37° 41'	36° 53'	36° 53'	36° 56'
Altitude (m):	1000	600	900	700	300	300	75	60	60	300
Size/Discharge (m^3/sec):	0.608	0.148	0.019	0.754	0.068	0.003	0.009	0.100	0.109	0.157
B.O.D.$_5$:	0.33	1.33	1.00	0.83	5.93	5.86	6.26	6.83	5.53	4.83
Vegetation:	Beech (red + silver)	Podocarp-hardwood	Beech (silver)	Hardwood	Podocarp-hardwood	Podocarp-hardwood	Podocarp-hardwood	P-hardwood	P-hardwood (+ mixed scrub + kauri)	P-hardwood
pH:	7.5	8	7.5	7.4	7.7	7.4	7.6	7.7*	7.6	6.8**
Temperature (C):	11.0	12.5	16.0	13.0	15.0	15.0	16.0	15.0	17.5	13.0
No. spp. (water)	18+	16	15+	15+	11	14+	12+	17+	20+	18+
No. spp. (lvs)	10+	14	13+	11	15	17	11	15	10	9
Total spores/l:	2591	896	671	2475	1738	968	2530	3402	1362	4001
Total spores/g:	137852	63486	34081	838384	1366827	740424	1409147	1613473	718764	315314
A. acuminata	20 /2	/2		1 /<1	1 /<1	2 /<1	/<1	4 /<1	9	6 /<1
Alatospora sp.	5 /1	1 /1		1 /<1	3 /<1	2 /<1	3 /1	5 /1	1 /1	/<1
A. crassa+long.	2+0	5+0	3+0	<1		2+0			0+1	0+1
A. filiformis		4 /2	7 /16	3 /2	/<1		/<1	1 /1	<1 /<1	5 /12
A. tetracladia	4 /<1		8 /4	3 /<1						/1
C. sphagnorum						/<1				/1
C. filicladia										
Ceratosporium sp										
C. aquatica		1	2 /1	3 /<1	2	5 /<1	/<1	1 /<1	<1	<1
C. longibrach.	3	1 /9	3 /<1	<1 /<1	5 /<1	9 /<1	3 /1	1	<1	2
Culic. aquatica									1	
D. aquatica								/<1	2 /<1	
E. microaquatica					1		<1			
Filosporella sp. 2					8 /2	2 /<1	11 /5	16 /2	17 /13	
F. penicill.	2	5	5							
F. eccentrica	<1			/2						
Gyoerffyella sp.										
H. stellatacula										
H. campanulata										1
L. uniinflata										

Appendix 15 cont.

	Waipakahi trib.		Hapuawhenua		Reid's Ck		Waihora str		Rangitukia		Rangitukia 2		Hakarimata		Swanson str		Cascades str		Nihotupu	
H. lugdunensis			1																	
L. aquatica	3	/1	9		2	/4	3	/4		/1		/<1			<1	/4	1	/<1		
L. ovalis			77	/1					2	/<1	9	/31	7	/3	7	/4	5	/<1	49	/68
Lunulospora spp	15	/9	35	/23	43	/28	16	/22	69	/93	57	/61	48	/86	44	/87	36	/84	8	/19
Mycocentrosp. sp																			1	
Pleuropedium sp.																				
T. gracilis					2															
T. elegans	8	/35	5	/10	2	/27	9	/18		/<1										
T. marchalianum	1		11	/10					6	/<1	2			/<1	1	/1	1	/<1		
T. setigerum				/24						/<1		/<1				/<1				/2
Tetracladium sp. 1				/14			<1							/<1						
Tetracladium sp 2																				
T. aquatica												/<1								
T. chaetocladium	1		1	/1	2	/1	1	/1		/1	2	/1	<1	/2	<1	/<1			<1	/<1
T. splendens						/1									1		5	/<1	1	/<1
T. acuminatus																	<1			
T. myrti		/<1		/<1		/<1														
Triposporina sp																				
V. elodeae																				
Unident. sp. 1									3	/<1		/5	21	/<1	6	/<1	6	/1		
Unident. sp. 2																				
Unid. s/r 1(2)	16	/46	9	/2			49	/51		/<1	2	/<1	<1		<1	<1	5		10	/1
Unid. s/r 1(3)	10	/3			5	/14				/<1	2	/<1	<1		2				1	
Unident. s/r 2	2		2			/2							<1							
Unident. s/r 4				/2							4				1				1	
Unident. s/r 10					2				1				2		1		1			
Unident. s/r 12												/<1								
Unident. s/r 14																				
Unident. s/r 15																				
Unident. s/r 18																				
Unident. s/r 19																				
Unident. s/r 20																				/<1
Unident. s/r 21																				
Unident. s/r 22	1		1		2		<1													
unid. tetrarad	1														<1		4			
unid. sigmoids					3		9				2		<1		10				12	

*Filter station upstream measured 6.7 -7.1 in 1980

**Monthly measurements in 1981 ranged 5.7 -6.7 (Aimer, 1982)

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