This is to certify that the

thesis entitled

Aquatic Hyphomycetes and Aquatic
Oomycetes As Decomposers of Leaves In A Northern
Michigan Stream

presented by

Deborah A. Repert

has been accepted towards fulfillment
of the requirements for

Master's degree in Zoology

Thomas M. Burton
Major professor

Date 12/17/93

O-7639

MSU is an Affirmative Action/Equal Opportunity Institution

AQUATIC HYPHOMYCETES AND AQUATIC OOMYCETES AS DECOMPOSERS OF LEAVES IN A NORTHERN MICHIGAN STREAM

By

Deborah A. Repert

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Zoology

1993

ABSTRACT

AQUATIC HYPHOMYCETES AND AQUATIC OOMYCETES AS DECOMPOSERS OF LEAVES IN A NORTHERN MICHIGAN STREAM

By

Deborah A. Repert

The purpose of this study was to identify species of aquatic oomycetes and aquatic hyphomycetes on speckled alder (*Alnus rugosa*) leaves submerged in the Ford River of Michigan, compare their distribution in various current regimes, determine their successional pattern on leaves and compare the enzyme activity of the predominant species. The predominant species found colonizing submerged leaves were the hyphomycetes *Tetracladium marchalianum* de Wildeman, *Lunulospora curvula* Ingold, and *Triscelophorus monosporus* Ingold and the oomycetes *Saprolegnia ferax* (Gruith.) Thuret, *Achlya bisexualis* Coker and A. Couch and *Dictyuchus monosporus* Leitgeb. Both classes of fungi exhibited a core group of species that dominated the decomposition period with rarer species occurring sporadically. There was no apparent difference between pool, run and riffle sites with regard to species occurrence. Individual 28 day *in vitro* growth studies using leaf discs as the carbon source were conducted for each of the predominant hyphomycete and oomycete species noted during the field distributional survey study described above. All species were able to produce enzymes capable of degrading plant cell wall components, such as amorphous cellulose, crystalline cellulose, pectin, and polygalacturonic acid but the quantity of each enzyme produced differed among species. Generally, the hyphomycete species exuded greater amounts of the enzymes analyzed.

ACKNOWLEDGEMENTS

I would like to thank my major professor, Dr. Thomas M. Burton, for his helpful advice and support throughout the development and completion of the study, and also for allowing me to use the Ford River nutrient, chemical and physical data from the ELF project in which he was involved. I would also like to express my sincere appreciation to my committee members Dr. Alvin L. Rogers, Dr. Donald J. Hall, and Dr. Michael J. Klug for invaluable ideas and constructive criticisms. Dr. Rogers generous offer of laboratory space, chemicals and guidance spurred this research project.

I would like to thank Dr. E. S. Beneke for help in identifying fungal species and Dr. Hammerschmidt for loaning me enzyme substrates. Dr. Margaret Carreiro, Dr. Robert Sinsabaugh, Dr. William Cooper, Dr. Jean Stout and Dr. Tesfaye Belay provided helpful input at various stages of my thesis. I thank Sue Eggert, Dr. Dennis Mullen, Bob Stelzer and Valerie Brady for assisting with sampling and photography.

TABLE OF CONTENTS

CHAPTER 1 - Stream Study

Review of Literature

3. Environmental Influences

Materials and Methods

Results

2. Species Frequency of Occurrence

3. Leaf Decomposition Data

LIST OF TABLES

LIST OF TABLES - Continued

LIST OF FIGURES

LIST OF FIGURES - Continued

CHAPTER 1 : Stream Study

INTRODUCTION

Within every river system exists a wide variety of habitats from which aquatic organisms may choose. Several biological, physical, and chemical parameters have been shown to affect the occurrence, distribution, and diversity of members of the aquatic community (Kaushik and Hynes 1970). The success of an individual species will be determined by the suitability of the particular conditions. Some organisms are able to adapt to a diversity of conditions while others are more sensitive to their surroundings and thus have a limited habitat range.

The distribution and role of aquatic fungi in streams have been intensely studied in the past 20-25 years since their implication in the degradation of organic matter. Much attention has been focused on this group in an attempt to shed light on the effect of the aquatic environment in which these organisms are submersed. Aquatic fungi are sensitive to pH, alkalinity, temperature, calcium and nutrient fluctuations. Quality and quantity of detrital inputs, stream turbidity and velocity, as well as invertebrate and bacterial communities also have been shown to influence the makeup of the aquatic fungal community (Barlocher 1980, Butler and Suberkropp 1986). Fungal communities may undergo successional shifts of species as a function of the changing seasons, suggesting that temperature plays an important part in the occurrence of fungi (Suberkropp 1984). Many of these fungi are also able to flourish at temperatures below which other organisms become inactive or perish.

The aquatic hyphomycetes have been the focus of much of the research aimed at answering questions surrounding the decomposition of stream organic matter. The aquatic oomycetes have received attention as saprophytes and decomposers of vertebrates and invertebrates in a wide variety of habitats, but especially ponds and small lakes. However, there is evidence for the occurrence of the aquatic oomycetes on leaves and twigs within the stream environment (Sparrow 1960, Willoughby and Collins 1966, Newton 1971, Park and McKee 1978).

Physical conditions within the stream may influence the choice of habitats by the oomycetes and the hyphomycetes. Oomycetes are thought to prefer slow-moving water near the edge of the stream or within pools while hyphomycetes are generally sampled where the water is swifter, such as riffles, as well as from the water column and within the foam that forms at barriers within the stream (Dick 1966, Webster and Towfik 1972, Shearer and Lane 1983, Shearer and Webster 1985). A great deal of research on both the aquatic hyphomycetes and the aquatic oomycetes has sought to describe the specific conditions required for success of species within each class (Perrott 1960, Dayal and Tandon 1962, 1963, Barlocher and Kendrick 1974, Ingold 1979, Khulbe 1980, Mer et al 1980, Webster and Descals 1981, Barlocher 1982, Misra 1982, 1983). However, it is difficult to characterize specific niches for each species of fungi due to the inherent uniqueness of each system.

The purpose of this portion of the study was to investigate the occurrence and distribution of aquatic hyphomycetes and oomycetes on leaves in the Ford River. An attempt was made to determine whether these two classes of fungi differed in their temporal and spatial occurrence and whether differing conditions of the stream affected the frequency and diversity of species on leaves in a stream within each class.

My hypotheses were that :

1) Aquatic hyphomycete communities are the dominant fungal decomposers in riffles while aquatic oomycete communities are the dominant decomposers in pools.

2) Aquatic oomycete communities exhibit a successional pattern during leaf decomposition that is similar to the pattern exhibited by hyphomycetes, that is, a core group of oomycete species dominates the leaf with predictable peaks as decomposition proceeds, while rarer species occur randomly during the leaf decomposition process.

3) Seasonal shifts in the aquatic oomycete community parallel those known to occur for aquatic hyphomycetes with increases during the fall and with low numbers during the summer months.

LITERATURE REVIEW

1. Community Structure

Aquatic hyphomycetes were first noted by deWildeman (1893-1895) in small lakes and ponds, usually growing on decaying leaves while Ingold (1942) was first to discover these organisms in streams. Research focusing on aquatic fungi in streams led to the conclusion that the aquatic hyphomycetes dominate the fungal community (Barlocher and Kendrick 1974, Ingold 1942, 1966, Nilsson 1964, Petersen 1962, 1963a,b). Much of this research dealt with discovering and identifying spores found in water, scum and leaf samples. Findlay and Arsuffi (1989) determined that fungi made up 82-96% of the microbial biomass on oak and elm leaves and 63-90% on sycamore leaves. Research on the succession of fungal species on leaves revealed that the community was actually dominated from start to finish by a small core group of species of aquatic hyphomycetes with rarer intermittent species occurring when substrate was sporadically available (Barlocher 1980, Sanders and Anderson 1978). Shearer and Webster (1985b) concluded that species responses to environmental factors may form a continuum with species that show extreme population changes as well as those which change minimally setting the limits of that continuum.

Hynes (1972) concluded that a variety of factors varied along a downstream gradient. Some of these factors include stream velocity, depth, vegetation, temperature, dissolved oxygen, pH and alkalinity. Several authors determined that the hyphomycete community composition was influenced by a variety of conditions. Triska (1970) and Iqbal and Webster (1973) showed that the community changed seasonally. Other researchers revealed a response to the physical and chemical quality of the stream (Barlocher and Rosset 1981, Eggenshwiler and Barlocher 1983). Research on the growth and sporogenic activity of the aquatic hyphomycetes revealed a need for running water in order for these processes to occur (Sanders and Webster 1980, Webster 1975, Webster and Towfik 1972). Chergui and Pattee (1988) compared the occurrence of these organisms in river channels, side arms, and oxbow lakes and concluded that aquatic hyphomycete prevailed in the faster channel water. Differences in the community were also apparent with comparisons of different riparian vegetation (Gonczol 1975, Iqbal and Webster 1977, Willoughby and Minshall 1975).

According to Willoughby (1962), the Saprolegniales are a "widely distributed and successful order". The occurrence of this order has been cited in a variety of habitats from lakes to ponds to streams and growing on substrata such as leaves, twigs and invertebrates. Sparrow (1960) and Dick (1968) noted the presence of members of the Saprolegniales in littoral habitats growing on seeds, twigs and insects. Willoughby (1962) associated Saprolegniales spore content with fish populations in England ponds. Of the lakes and ponds he surveyed, the genus *Saprolegnia* was the most abundant with species within the *Achlya* and *Aphanomyces* genera intermediate in occurrence and *Dictyuchus* and *Leptolegnia* species only occasional isolates. Studies by Hallett and Dick (1981) support this observation. Other researchers of water moulds (Harvey 1952, Hasija and Batra 1978) noted the appearance of these organisms in clear, unpolluted waters but not in waters influenced by pollution or residential areas.

Chamier and Dixon (1982a) in a field study of the aquatic hyphomycetes found that certain species were apparent only when a specific sampling method was employed while others were identified in a diversity of situations. Suberkropp and Klug (1976) also noted that the successional patterns were affected by the specific stream mycoflora, sampling method, and methods used in the determination of the predominant species. Sparrow (1960) and Perrott (1960) found a similar phenomena with the aquatic oomycetes. They discovered there was a close relationship between the substrata used for baiting and the fungi isolated.

2. Community Function

The chain of events during leaf breakdown involve the leaching of soluble compounds (Nykvist 1963), microbial colonization, then invertebrate attack (McDiffet 1970, Peterson and Cummins 1974, Sedell *et al* 1975, Winterbourn 1978). The avenues open to stream fungi include the exploitation of the substrate for energy, preying upon other organisms present or use of their waste products, or simply use of the substrate as a holdfast. The presence of aquatic hyphomycetes on leaves led to speculations on the role of these organisms during decomposition. Ideas put forth by Barlocher and Kendrick (1983) that hyphomycetes were dominant decomposers during at least some stages of decomposition, were typical of the prevailing sentiment regarding these fungi.

Research on the role of the aquatic hyphomycetes during decomposition led many to believe that they were the predominant members of the microbial community during the initial stages of leaf degradation (Barlocher and Kendrick 1974, Kaushik and Hynes 1971, Suberkropp and Klug 1976, Triska 1970). Mechanistically fungi were important during decomposition through fungal production or enrichment of the substrate via cells and secretions and also through catalysis or the breakdown of the leaf components into forms usable by invertebrates (Anderson and Sedell 1979, Barlocher and Kendrick 1981, Cummins and Klug 1979). Aquatic hyphomycetes were shown to possess the enzymes necessary to metabolize components of the plant (Chamier and Dixon 1978a, b, Suberkropp and Klug 1976). A study of the aquatic hyphomycetes during leaf decomposition in the Rhone River by Chergui and Pattee (1988) revealed similar results and led to the conclusion that the transversal gradient in the rate of decomposition was determined by physical and chemical gradients, invertebrate attack and aquatic hyphomycete activity.

Research on the aquatic oomycetes in the early 1960's, especially by Willoughby (1962), revealed the ability of these organisms to decompose complex organic material. Their role as saprophytes in the decomposition of fish was apparent. The ability of some species within the oomycete order to produce the cellulase enzyme led to the supposition that these organisms may also play a part in the decomposition of stream organic matter such as leaves and twigs (Miele and Linkins 1974, Park and McKee 1978, Thomas and Mullins 1967 and 1969). Roberts (1963) found that species inhabiting acid waters tolerated a wider pH range and suggested these species were more likely to be involved in decomposition in acid waters, where bacterial numbers were less, than alkaline species in alkaline water due to competition by bacteria. Alkaline species therefore would be more likely to live on secondary products.

3. Environmental Influences

a. Temperature

Research on the temperature conditions for occurrence and optimal growth of the aquatic hyphomycetes suggested that the aquatic hyphomycete, *Tetracladium marchalianum,* dominated the fungal community in temperate climates (Barlocher and Kendrick 1974, Shearer and Lane 1983, Suberkropp and Klug 1976) while *Lunulospora curvula* was the dominant decomposer in the tropics (Ingold 1979, Thomas *et al* 1989, Sridhar and Kaveriappa 1989). Laboratory studies of a variety of species determined the temperature for maximum growth of each species was related to the occurrence of individual species in the field (Ingold 1979, Nilsson 1964, Suberkropp and Klug 1976, Webster *et al* 1976). Adaptation to specific temperature ranges resulted not only in temporal variation in the occurrence of individual species but also to seasonal variation in the numbers of conidia produced with a peak during leaf fall (Barlocher and Rosset 1981, Chamier and Dixon 1982, Iqbal, Bhatty and Malik 1979, Iqbal and Webster 1973, Shearer and Webster 1985).

Field studies, however, showed that while occurrence of aquatic hyphomycete species partially depended on the stream temperature it was also influenced by seasonal changes and the large input of detrital material (Sridhar and Kaveriappa 1989, Suberkropp 1984). Shearer and Webster (1985b) also pointed out the difficulty in attributing more importance to either temperature or quality and quantity of substrate since both change at the same time. Also, the magnitude of the variation between species differed depending on the individual species, making wide generalizations inappropriate.

Petersen (1910) first noticed a periodicity in the aquatic oomycetes and determined that their growing season began in the spring and lasted until mid-fall. Several researchers concluded that the winter months were the most suitable time for growth of aquatic fungi (Dayal and Tandon 1962 & 1963, Forbes 1935, Manoharachary and Ramarao 1981, Misra 1982 & 1983, Srivatava 1967, Waterhouse 1942). Khulbe and Bhargava (1977) studied the occurrence of water moulds in subtropical as well as temperate lakes of India and determined that the greatest frequency occurred during the summer and autumn in temperate climates and during the spring and autumn in subtropical regions. Mer *et al* (1980) found that the maximum number of species was associated with the rainy season (July to September). Studies by Perrott (1960) and Dick and Newby (1961) showed there to be two periods of maximum growth, one in early spring and one in late autumn. In a later study, Hallett and Dick (1981) noted three periods of maximum propagule availability.

Coker (1923) noted a temporal successional pattern with certain species occurring more frequently during the warmer months and others during the colder months. A comparison of the genera *Achlya* and *Saprolegnia* by Lund in 1934 led him to the conclusion that species of *Achlya* thrived in cold water while *Saprolegnia* species were not as dependent on temperature conditions. A study by Willoughby (1962) led him to the conclusion that spore content of lakes in Blelham Tarn was related to seasonal rainfall. Heavy rainfall happened to coincide with the autumn leaf fall which is an important factor in the stream ecosystem. Dayal and Tandon (1963) reported that the oospores of aquatic oomycetes were dormant during the warmer summer months and began germinating when the temperature began to drop in August and September and reached a maximum in spring. He concluded that this increase in productivity during autumn was associated with an increase in nutrients and dissolved oxygen content of the water. Other researchers classified particular species into seasonal categories (Gupta and Mehrotra 1989, Roberts 1963) and found that a number of species were prevalent throughout the year.

b. Alkalinity and pH

In the 1980's research began focusing on the chemical composition of the stream water and its relationship to the productivity of fungal species.

Aquatic hyphomycete diversity was shown by Barlocher (1987), Barlocher and Rosset (1981), and Wood-Eggenschwiler and Barlocher (1983) to be greater in slightly acidic, softwater streams than in more alkaline streams. Barlocher and Rosset (1981) determined there were twice as many species in soft water as in hard water. They postulated this was due to decreases in invertebrate feeding, flood incidences, or sedimentation as a result of lower suspended loads which otherwise would inhibit the supply of oxygen to the leaf surface. They also determined that there was no correlation between the optimal pH for these organisms in the laboratory and their occurrence in the stream. Research by Chamier and Dixon (1983) conflicted with the studies discussed above. They found that increasing Ca^{2+} levels in the laboratory led to increased growth of the hyphomycetes as well as the maceration of leaf material. Suberkropp (1991) also found that there was greater fungal biomass, activity and numbers of species on leaf discs in hard water as compared to soft water.

Similar research on aquatic oomycetes showed that a correlation between pH and species occurrence was variable depending on each particular species (Khulbe 1980, Lund 1934, Sparrow 1960). Some species are able to tolerate a wide range in pH while others are restricted to a very narrow range. Roberts (1963) studied the distribution of Saprolegniales members in a variety of water habitats ranging from acid bogs to alkaline lakes. Based on his findings he categorized species as acid ($pH<5.2$), alkaline ($pH>7.8$) or neutral (pH5.7-7.8) species and found the greatest number of species within the pH range 5.6-7.4. Khulbe (1980) conducted a study of alkaline waters and found that even within this pH category species had wide, moderate or narrow pH ranges. Weston (1941), however, concluded that environmental factors such as light, pH, and chemical composition are not directly correlated with the distribution of aquatic oomycetes.

c. Invertebrates

Interest in the influence of fungi on the invertebrate community and vice versa developed in the 1970's. Researchers questioned whether the palatabiltiy of a leaf species increased due to the fungal presence or the affect that presence had on the leaf itself. Barlocher and Kendrick (1973a, b, 1975) determined that *Gammarus pseudolimnaeus* profited from both the microbial cells as well as the change in leaf composition as a result of fungal colonization. They also concluded that individual species selection was not related to the nutritional value of the fungi. A comparison of different species of aquatic hyphomycetes and their affect on two species of caddisflies by Arsuffi and Suberkropp (1986) showed that larval caddisflies had a distinct preference for certain species of fungi which, along with conditioning time, determined their consumption and growth rates.

Marcus and Willoughby (1978) studied the feeding preference of the aquatic invertebrate, *Asellus aquaticus,* when given the choice of the oomycete *Saprolegnia* spp., or the hyphomycete, *Lemonniera aquatica,* and concluded that growth of the invertebrate was better when fed the oomycete than the hyphomycete. Presumably, the higher proportion of hyphal walls in the hyphomycete make it more difficult to digest. However, the invertebrate grew just as well on oak (*Quercus*) leaves as on the oomycete. Very little research has focused on the interaction of invertebrates and oomycetes on leaves.

Alternatively, invertebrates affect the fungal community not only through the grazing of the organism but also by the destruction of the fungal habitat thus acting as competitors for space as well as predators (Barlocher 1980). The long term affect of this activity on the fungal structure is the prevention of species that may be late colonizers and therefore lower species richness. This activity has been associated with water hardness due to the diminished presence of invertebrates in soft water.

d. Vegetation

Studies of decomposition and the associated microorganisms have emphasized the importance of autumn leaf fall and the concommitant biomass input into streams (Anderson and Sedell 1979, Vannote *et al* 1980). Factors such as cuticle thickness, nitrogen and phosphorus leaf concentration, and plant compounds (e.g. phenols) that may inhibit microorganisms or macroinvertebrates affect the rate of decomposition of particular leaf species (Covich 1988, Meyer 1980, Sparling *et al* 1981, Suberkropp *et al* 1976, Taylor *et al* 1989, Valiela 1979, Webster and Benfield 1986). Wood-Eggenshwiler and Barlocher (1983) found there was a significant correlation between the concentration of fungal spores and the type and frequency of vegetation influencing detrital input. In a study aimed at determining the effects of leaf size and decay rate on the aquatic hyphomycete community, Barlocher and Schweizer (1983) showed that there is a temporal influence on the number of species that colonize a particular resource and the success of a species depends on its arrival time and expediancy in reproduction. Also, the initial area of the leaf is only a rough estimate of the number of individuals likely to colonize it.

Other researchers focused on the significant input of fresh, green leaves into streams during the summer season and the impact on the decomposer community (MacArthur *et al* 1986, Risley and Crossley 1988, Stout *et al* 1985). Decomposition of green leaves during the summer was found to be faster than decomposition of senescent leaves during the fall (MacArthur *et al* 1986, Stout *et al* 1985). This finding was attributed to higher nutrient concentrations in green leaves. However, Leff and MacArthur (1990) found that although the C to N ratio was higher in senescent leaves than green leaves, decomposition was not significantly different when leaves were incubated under the same physical conditions. Comparisons of fungal communities on fresh (green) leaves versus senescent leaves suggested that leaching of soluble substances from green leaves during the first few days of submergence in the water did not occur and that fungal colonization and growth may actually be inhibited (Gessner and Schwoerbel, 1989; Barlocher, 1990). Leff and MacArthur (1990) concluded that microorganisms are adapted to the natural timing and type of input and are limited in their capacity to respond to unusual conditions. Barlocher (1992) suggested that other microorganisms such as bacteria, oomycetes, and yeasts benefit from the retention of the soluble substances in fresh leaves. Leaf preference studies of aquatic oomycetes are scarce. Most research on these organisms centers on their existence in the water column or as parasites of fish.

METHODS AND MATERIALS

1. Site Description

The Ford River on the Upper Peninsula of Michigan is located in Dickinson County (Sec 18, T. 43 N., R. 29 W.). It is a 4th order, brown, hardwater stream. The stream bottom substrate consists mostly of cobble. The catchment area is composed mostly of early successional hardwood trees and the stream is lined primarily by speckled alder, *Alnus rugosa*, balsam poplar, *Populus balsamifera*, and quaking aspen, *Populus tremuloides*.

Collection sites were selected to maximize the degree of similarity. In the summer of 1990, two sites were chosen, previously designated as Ford Experimental (FEX) and Ford Control Downstream (FCD) on the Ford River and separated by approximately 8 km. Two subsites within each site were also chosen, a fast-flowing riffle and a slow-flowing pool, separated by approximately 10 m. Subjective measures of leaf and light input as well as objective measures of water velocity and depth were used as deciding factors in the selection of sites. The following summer of 1991, one moderate-flowing site was chosen at each of the two sites, FEX and FCD, due to no significant differences between the pool and riffle subsites at either site the previous year. Sites were again matched on the basis of water velocity, depth, and leaf and light input.

2. Summer 1990 Sampling Method

Fresh speckled alder, *Alnus rugosa* (Du Roi) Sprengel, leaves were collected from a tree bordering the river in 1990. Three leaves were stacked together into a leaf pack and immediately attached to each of 120 bricks with labelled rubber bands. Thirty leaf packs were placed at each site on five rows of bricks separated by 0.5 meters and with six bricks per row. Rows were placed approximately 1 meter from the shore. The experiment was conducted between July 29 and September 10, 1990. One leaf pack from each of the five rows was randomly selected, based on a random number table, at each site after exposure periods of 3, 7, 14, 21, 28, and 42 days. While in the field, two 9 mm discs were cut with a cork borer from the top leaf of each leaf pack and stored in whirlpak bags for transport to the lab. Current velocity was measured at the center of each leaf pack with a Gurley pygmy current meter prior to the removal of the leaf pack and brick from the river. Water temperature and depth were recorded at each sampling time with a hand-held thermometer and meter stick, respectively.

The two 9 mm leaf discs from each leaf pack were returned to the laboratory with one used for hyphomycete detection and the other for oomycete detection. The hyphomycete disc was rinsed with distilled water, placed in a jar containing 50 ml distilled water, covered with parafilm and aerated at room temperature. The water and the leaf disc were checked every two days for approximately 10 days for spores under a compound light microscope. Resulting spores were mostly identified using Ingold (1975) and Peterson (1962, 1963a, 1963b). The oomycete disc was rinsed with distilled water and placed in a petri dish containing 25 ml distilled water and 5-7 half hemp seeds. Colonies growing on the hemp seeds were identified under a compound light microscope. Every 7-10 days the petri dish water was renewed and fresh hemp seeds were added. Petri dishes containing the oomycete leaf discs were also examined for hyphomycete spores that may be present. Oomycete species were identified using Coker (1923), Coker and Matthews (1937), Johnson (1956), Sparrow (1960) and Wolf (1944).

The remainder of the top leaf from each leaf pack was passed through a leaf area meter to determine the final area. The initial area of each leaf was determined subjectively by reconstruction based on the final appearance of the leaves. Leaves were traced onto construction paper, cut out and passed through the leaf area meter. The area of a leaf disc was determined by averaging values obtained from several leaf discs passed through the meter. The area of two leaf discs was added to the final leaf area. The final whole leaf area was divided by the initial leaf area and multiplied by 100 to provide an average percent leaf area remaining at each sampling date.

3. Fall 1990 Sampling Method

A second sampling routine was conducted from September 9, 1990 to December 10, 1990. The sampling set-up and identification procedure were similar to the previously described experiment except only ten leaf packs per site were placed in the river and leaf area and water velocity was not measured. Three leaf packs were retrieved after 27 and 48 days while four leaf packs were retrieved after 91 days of exposure in the river.

4. Winter 1990-91 Sampling Method

Beginning October 29, 1990 a six week sampling schedule was employed. The first period ended December 10, 1990. Four leaf packs were placed in the river at each site on day one of the interval and all four were collected at the end of the six weeks, replaced by four new leaf packs to begin the next cycle. Winter sampling differed from the summer and fall sampling in that leaves from speckled alder trees gathered just after abscission were utilised as the bait and water velocity could not be measured. Leaf area also was not measured.

5. Summer 1991 Sampling Method

The 1990 sampling procedure was changed in 1991 to assure that the entire leaf remained in aerobic conditions and was available for fungal colonization. The leaf packs used in 1990 could have led to anaerobic conditions in the leaf packs. Fresh leaves from one speckled alder tree were picked, dried at 40°C for 48 hours and weighed. Individual leaves were sandwiched between labelled tongue depressors, attached by the petiole to wooden dowels with wire and soaked in distilled water overnight . The tongue depressors were removed and dowels with the individual leaves were then suspended within a cage, 50 cm x 50 cm x 80 cm, enclosed by chicken wire and submerged in the stream. Cages were submerged in moderately flowing water at both FEX and FCD sites. Four leaves were randomly sampled at 3, 7, 14, 18, 21, and 22 days between July 12 and August 19, 1991. The experiment was repeated between August 16 and September 13, 1991 with sampling after 3, 7, 14, 21, and 28 days of stream exposure. During each sampling date stream flow at 30, 45, and 60 cm above the stream sediment surface was measured at each of the four corners of the cage. The water depth at each corner was also measured and stream water temperature was recorded at the time of sampling. Three of the leaves sampled were used in the identification of fungi while the fourth leaf was used to determine what area of leaf had been removed with the six leaf discs taken from the leaf at the various sampling dates. From each of the leaf samples used in the identification process, a total of six discs were cut, three of which were used for oomycete detection and three for hyphomycete detection. The six leaf discs and remaining leaf portion were dried separately at 40°C for 48 hours, weighed then ashed at 500°C for 30 minutes and weighed. The Ash Free Dry Weight (AFDW) of the leaf discs was divided by the total AFDW of the leaf and multiplied by 100 to provide a percent by which the weight of the other leaf samples (minus the six leaf discs used for analysis) could be multiplied to obtain an estimate of the total leaf remaining. The remainder of the leaf was dried at 40°C for 48 hours, weighed then ashed at 500°C for 30 minutes to determine the ash free dry weight. The procedure for identification of the fungal organisms did not differ from that described for the previous summer.

6. Species Selection and Maintenance

Selection of species was based on the most predominant species within each class common to both riffle and pool sites during the first sampling period in the summer of 1990 from July 29 to September 10. Percent frequency of a species was determined by a positive or negative occurrence on each of the five leaves sampled. Thus, results pertain only to the dominant summer flora. However, samples collected at other times throughout the year provide an idea of the seasonal dynamics of the two groups. Three species of hyphomycetes and three species of oomycetes were chosen for study.

7. Monitoring of Chemical/Physical/Biological Parameters

Several physical and chemical stream parameters were monitored on a regular basis. Stream pH was measured using an Orion Model 407A Ionalyzer. Hardness and alkalinity measurements were determined by using the EDTA and sulfuric acid titration methods, respectively, as described in APHA, 1980. Measurements of dissolved oxygen were obtained following the sodium azide modification of the Winkler method described in APHA, 1980. Turbidity was measured with a Hach Model 2100A Turbidimeter. A YSI Model 31 Conductivity meter was used to measure conductivity. Concentrations of select nutrients including ammonia, chloride, nitrate, nitrite, orthophosphorus, silica, total nitrogen and total phosphorus, were measured using a Technicon Autoanalyzer II and following the methods described by U.S.E.P.A., 1979. The phenate method was employed for measuring ammonia. Chloride concentration was determined with the ferricyannide method. The cadmium reduction method was used to determine nitrate concentration and the sulfanilic acid/NED dihydrochloride method to measure nitrite concentration. Orthophosphorus was measured using the ascorbic acid reduction method. To determine silica concentration, the molybdosilicate method was employed. Water and air temperatures were measured using a hand held thermometer. Measurements were taken biweekly from June to September and once every six weeks for the remainder of the year. Discharge was calculated weekly throughout the summer months using a Gurley pygmy current meter to measure water velocity at each of 20 points across a stream width transect.

8. Statistical Analyses

Values for the leaf area remaining during the summer of 1990 and the leaf weight remaining during the summer of 1991 were arcsin(h)*sqrt transformed for statistical analysis. An ANOVA test was used to determine differences between sites for leaf area remaining in 1990, leaf weight remaining in 1991 and frequency of occurrence of species in 1990 and 1991. During the summer of 1990, comparisons included FEX Pool vs FEX Riffle, FEX Pool vs FCD Pool, FEX Riffle vs FCD Riffle, and FCD Riffle vs FCD Pool. Comparisons during the summer of 1991 were between the FEX and FCD sites. Paired t-tests and correlations were also conducted for each of the comparisons.

RESULTS

1. Species Lists

The aquatic hyphomycete and aquatic oomycete species identified on leaves in the Ford River during the 1990 and 1991 field seasons are listed in the appendix (Table A-1). In 1990, 12 species of aquatic hyphomycetes and 14 species of aquatic oomycetes were identified. In 1991, 21 species of hyphomycetes and 17 species of oomycetes were identified. More species identified in 1991 vs. 1990 may be a result of differences in sampling methods, that is, leaf packs vs. suspended leaves as well as fresh vs dried leaves. In general, species that were observed in 1991 but not 1990 were rarer species that occurred sporadically throughout the sampling periods. An increase in the number of leaf discs per leaf sampled in 1991 may have been more efficient at sampling the fungal community.

2. Species Frequency of Occurrence

a. Summer 1990

The frequency of each species identified was calculated for the four sites during the three 1990 sampling periods (Table 1). More detailed tables of each species on each date are given in the appendix (Tables A-2 to A-4). The hyphomycetes : *Lunulospora curvula, Tetracladium marchalianum,* and *Triscelophorus monosporus* and the oomycetes : *Achlya bisexualis, Dictyuchus monosporus,* and *Saprolegnia ferax* were the predominant fungal species of each class during the 1990 sampling period. The hyphomycete and oomycete

Table 1 List of species observed and frequency of occurrence at each site during 1990.

	July 29 to Sept 10				Sept 9 to Dec 10				Oct 29 to Dec10			
	FEX		FCD		FEX		FCD		FEX		FCD	
Species observed	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool
HYPHOMYCETES												
Alatospora acuminata	0	0	0	0	0	8	8	17	100	50	0	50
Anguillospora longissima	0	0	0	0	23	36	55	33	25	0	25	50
Articulospora grandis	0	0	0	0	0	0	0	8	0	0	0	50
Articulospora tetracladia	10	10	10	7	11	10	10	7	0	75	0	0
Centrospora acerina	3	0	0	0	0	11	8	0	0	25	0	0
Clavariopsis aquatica	0	0	0	3	8	8	8	17	0	0	0	0
Flagellospora curvula	7	7	3	7	11	11	11	19	0	0	25	75
Heliscus lugdunensis	0	0	0	3	0	22	11	0	0	25	0	0
Lunulospora curvula	23	17	17	13	8	0	0	22	0	0	0	0
Tetracladium marchalianum	7	10	20	17	66	33	33	33	75	75	75	50
Tricladium angulatum	0	0	0	0	28	25	17	8	25	25	0	25
Triscelophorus monosporus	23	17	40	37	50	28	50	30	0	0	25	0
OOMYCETES												
Achlya americana	3	7	0	0	8	0	0	0	0	0	0	0
Achlya bisexualis	30	40	20	23	8	36	0	28	0	0	0	0
Achlya caroliana	0	3	0	0	0	8	0	0	0	0	0	0
Achlya conspicua	0	7	0	0	0	0	0	0	0	0	0	0
Achlya flagellata	0	0	0	3	0	0	0	0	0	0	0	0
Achlya hypogyna	3	0	0	0	0	0	0	0	0	0	0	0
Achlya klebsiana	0	13	0	0	0	0	0	8	0	0	0	8
Achlya polyandra	0	10	0	0	0	0	0	0	0	0	0	0
Achlya racemosa	0	7	0	0	0	0	0	0	0	0	0	0
Dictyuchus monosporus	13	13	0	3	0	11	8	8	0	0	0	0
Saprolegnia diclina	0	0	0	3	0	8	0	0	25	0	0	0
Saprolegnia ferax	33	23	40	33	8	22	19	0	0	25	0	0
Saprolegnia parasitica	0	7	3	3	0	0	0	8	50	50	25	100
Saprolegnia terrestris	3	0	0	0	0	0	0	0	0	0	0	0

fungi exhibit different seasonal trends. For most of the hyphomycete species, there was in an increase in frequency of occurrence of some species during the autumn as well as the appearance of several other species not evident during the summer months. The most pronounced changes were the appearance of *A. longissima, A. acuminata,* and *T. angulatum* and the decline of one of the most predominant summer species, *L. curvula*. Unlike *L. curvula*, the species *Triscelophorus monosporus, Clavariopsis aquatica*, and *Flagellospora curvula* showed peaks in frequency of occurrence early in autumn and *Alatospora acuminata* and *Tetracladium marchalianum* reached peaks in dominance during the late fall, early winter period as water continued to cool and as leaf detritus became more and more conditioned in the stream.

Unlike the hyphomycetes, most oomycete species declined as the stream temperature decreased. *Saprolegnia parasitica*, a minor component of the summer and fall flora, was one of the few oomycetes to be able to survive into the early winter months.

Frequency data were compared between FCD and FEX sites and riffle and pool subsites for total species observed, only hyphomycete species, only oomycete species, and the selected species alone for the July 29 to September 10 (Table 2) and September 9 to December 10, 1990 (Table 3). Correlation results of the first experiment were significant between sites, except when FEX and FCD riffle and FEX and FCD pool sites were compared for the selected species alone. Spearman correlations of all species and selected species confirm these results (Table 4). Sites were not significantly different according to paired t-tests, except when FEX and FCD pool sites were compared for the oomycete species. These results suggest there is more variation between sites than within habitats at a given site. There was a lesser degree of correlation during the period between September 9 and December 10, 1990, also

Table 2 Paired t-values and correlation coefficients for the frequency of occurrence of species at FEX and FCD sites for July 29 to September 10, 1990.

Comparison	t-value	probability	correlation coefficient
Selected Species n=6			
FEX Riffle vs Pool	0.502	0.637	0.752*
FCD Riffle vs Pool	1.117	0.315	0.975**
FEX Riffle vs FCD Riffle	-0.258	0.807	0.563
FEX Pool vs FCD Pool	-0.178	0.866	0.320
Total Species n=26			
FEX Riffle vs Pool	-1.217	0.235	0.851**
FCD Riffle vs Pool	-0.154	0.879	0.983**
FEX Riffle vs FCD Riffle	0.166	0.870	0.867**
FEX Pool vs FCD Pool	0.947	0.353	0.718**
Hyphomycete Species n=12			
FEX Riffle vs Pool	1.301	0.220	0.967**
FCD Riffle vs Pool	0.329	0.748	0.984**
FEX Riffle vs FCD Riffle	-0.732	0.480	0.861**
FEX Pool vs FCD Pool	-1.193	0.258	0.846**
Oomycete Species n=14			
FEX Riffle vs Pool	-1.912	0.078	0.841**
FCD Riffle vs Pool	-0.528	0.606	0.981**
FEX Riffle vs FCD Riffle	1.186	0.257	0.906**
FEX Pool vs FCD Pool	2.302	0.039*	0.770**

* $p < 0.05$
** $p < 0.01$

Table 3 Paired t-values and correlation coefficients for the frequency of occurrence of species at FEX and FCD sites for September 9 to December 10, 1990.

Comparison	t-value	probability	correlation coefficient
Selected Species n=6			
FEX Riffle vs Pool	0.175	0.868	0.530
FCD Riffle vs Pool	-0.224	0.832	0.328
FEX Riffle vs FCD Riffle	0.774	0.474	0.824*
FEX Pool vs FCD Pool	0.256	0.808	0.439
Total Species n=25			
FEX Riffle vs Pool	-0.775	0.446	0.676**
FCD Riffle vs Pool	-0.139	0.891	0.668**
FEX Riffle vs FCD Riffle	-0.165	0.870	0.772**
FEX Pool vs FCD Pool	0.612	0.546	0.657**
Hyphomycete Species n=12			
FEX Riffle vs Pool	0.250	0.807	0.730*
FCD Riffle vs Pool	0.373	0.717	0.701*
FEX Riffle vs FCD Riffle	-0.114	0.911	0.718*
FEX Pool vs FCD Pool	-0.047	0.964	0.475
Oomycete Species n=14			
FEX Riffle vs Pool	-1.834	0.090	0.666*
FCD Riffle vs Pool	-0.687	0.504	-0.067
FEX Riffle vs FCD Riffle	-0.164	0.873	0.446
FEX Pool vs FCD Pool	1.173	0.262	0.717**

* $p < 0.05$
** $p < 0.01$

Table 4 Spearman Correlation Coefficient test of the frequency of occurrence of total species and selected species for the 1990 and 1991 summer sampling periods.

Comparison	N	Rho Value	Significance
Summer 1990 - 1			
All species			
FEX Riffle vs FEX Pool	26	0.652	$p < 0.01$
FCD Riffle vs FCD Pool	26	0.856	$p < 0.01$
FEX Riffle vs FCD Riffle	26	0.745	$p < 0.01$
FEX Pool vs FCD Pool	26	0.575	$p < 0.01$
Selected species			
FEX Riffle vs FEX Pool	6	0.941	$p < 0.05$
FCD Riffle vs FCD Pool	6	0.971	$p < 0.02$
FEX Riffle vs FCD Riffle	6	0.522	NS
FEX Pool vs FCD Pool	6	0.493	NS
Summer 1990 - 2			
All species			
FEX Riffle vs FEX Pool	26	0.638	$p < 0.01$
FCD Riffle vs FCD Pool	26	0.473	$p < 0.02$
FEX Riffle vs FCD Riffle	26	0.685	$p < 0.01$
FEX Pool vs FCD Pool	26	0.503	$p < 0.02$
Selected species			
FEX Riffle vs FEX Pool	6	0.516	NS
FCD Riffle vs FCD Pool	6	0.406	NS
FEX Riffle vs FCD Riffle	6	0.647	NS
FEX Pool vs FCD Pool	6	0.600	NS
Summer 1991 - 1			
All species	38	0.732	$p < 0.01$
Select species	6	0.886	$p < 0.05$
Summer 1991 - 2			
All species	38	0.658	$p < 0.01$
Select species	6	1.000	$p < 0.01$

confirmed by Spearman's correlation. This was probably due to greater fluctuations in temperature over that time period. However, paired t-tests showed there were not significant differences between sites.

A two-way ANOVA, using the percent occurrence of the selected species as the dependent factor and site (FEX and FCD) and habitat (Riffle and Pool) as the grouping factors, showed there were no significant date, site or interaction effects, except a site effect for *Dictyuchus monosporus*, between the four sites (Table 5). These results were confirmed by Friedman's Chi^2 test . The percent occurrence of six of the more common species at each date and site during the July 29 to September 10, 1990 sampling period also suggests that there is more between site variation than within habitat variation at each site (Figures 1 and 2). The similarity within the hyphomycete and the oomycete communities between the four sites using Jaccard's and Sørensen's coefficient indicated a greater degree of similarity within the hyphomycete community than within the oomycete community during the first sampling period (Table 6). A similar pattern was evident in the second sampling period but the degree of similarity between each class was lower (Table 6).

b. Summer 1991

Total frequency of species occurrence was calculated for the hyphomycete and oomycete species observed on each sampling date during the July 12 to August 19, 1991 and August 16 to September 13, 1991 sampling periods (Table 7). Results for each date are given in tables A-5 to A-8 in the appendix. As in 1990, the predominant hyphomycete species were *Lunulospora curvula, Tetracladium marchalianum,* and *Triscelophorus monosporus*, with *Alatospora acuminata*, *Anguillospora longissima* and *Clavariopsis aquatica* also appearing frequently . The oomycetes *Achlya*

Table 5 Two-way ANOVA and Friedmans' Chi^2 test of selected species frequency on leaves at all sites from July 26 to September 10, 1990. Frequencies for the ANOVA were arcsin*sqrt transformed.

	Two-Way ANOVA			Friedmans' Test
Species	Site	Habitat	Interaction	Chi^2 value
Achlya bisexualis	0.012	0.012	0.421	1.545
Dictyuchus monosporus	9.265*	0.106	0.288	6.534
Saprolegnia ferax	0.281	0.281	0.450	4.200
Lunulospora curvula	0.335	0.187	0.055	1.345
Tetracladium marchalianum	2.387	0.196	0.196	5.824
Triscelophorus monosporus	0.034	1.378	0.656	1.688

* significant at $p \leq 0.05$

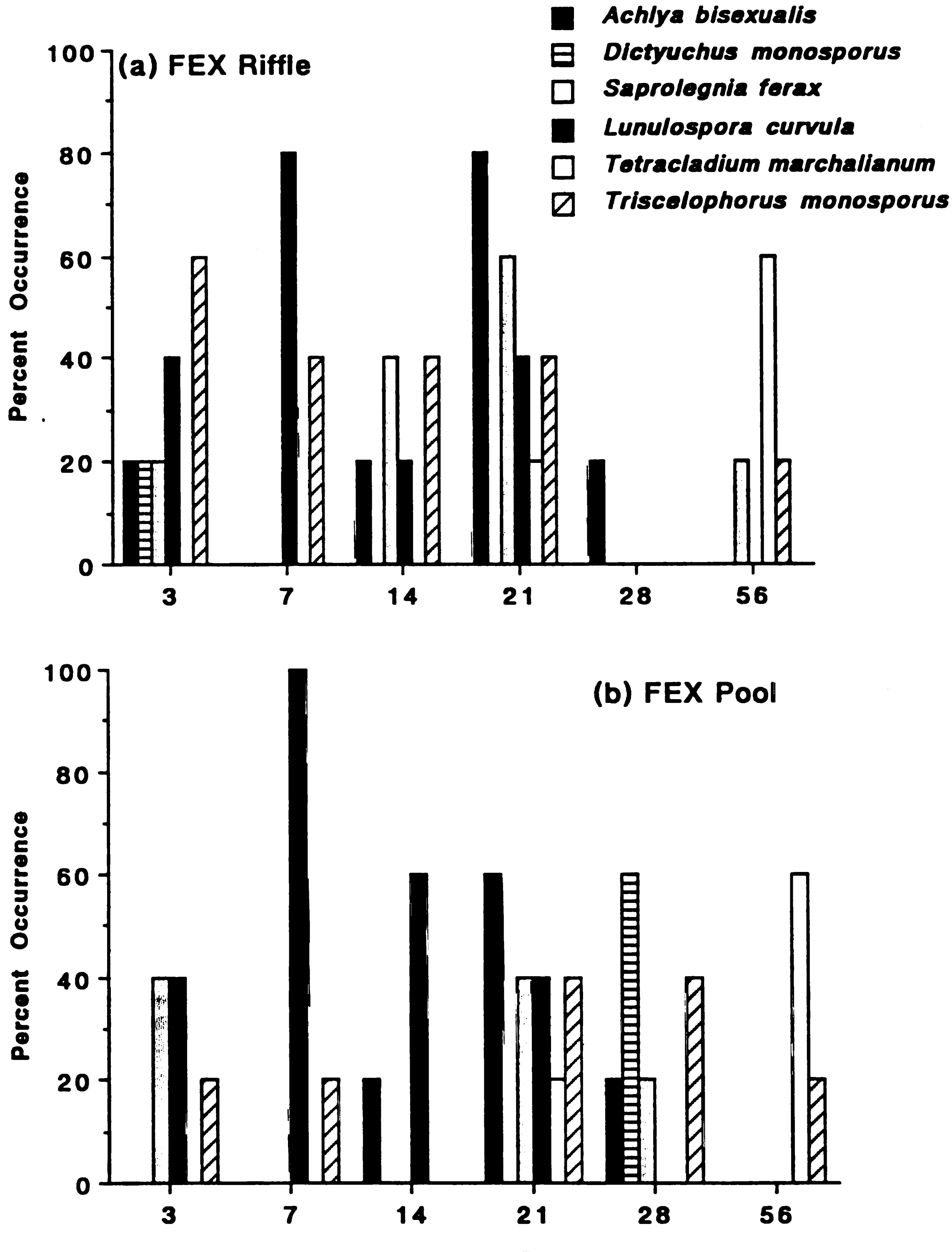


Figure 1 Percent Frequency of Occurrence of Select Species at FEX Riffle (a) and FEX Pool (b) sites from July 29 to September 10, 1990.

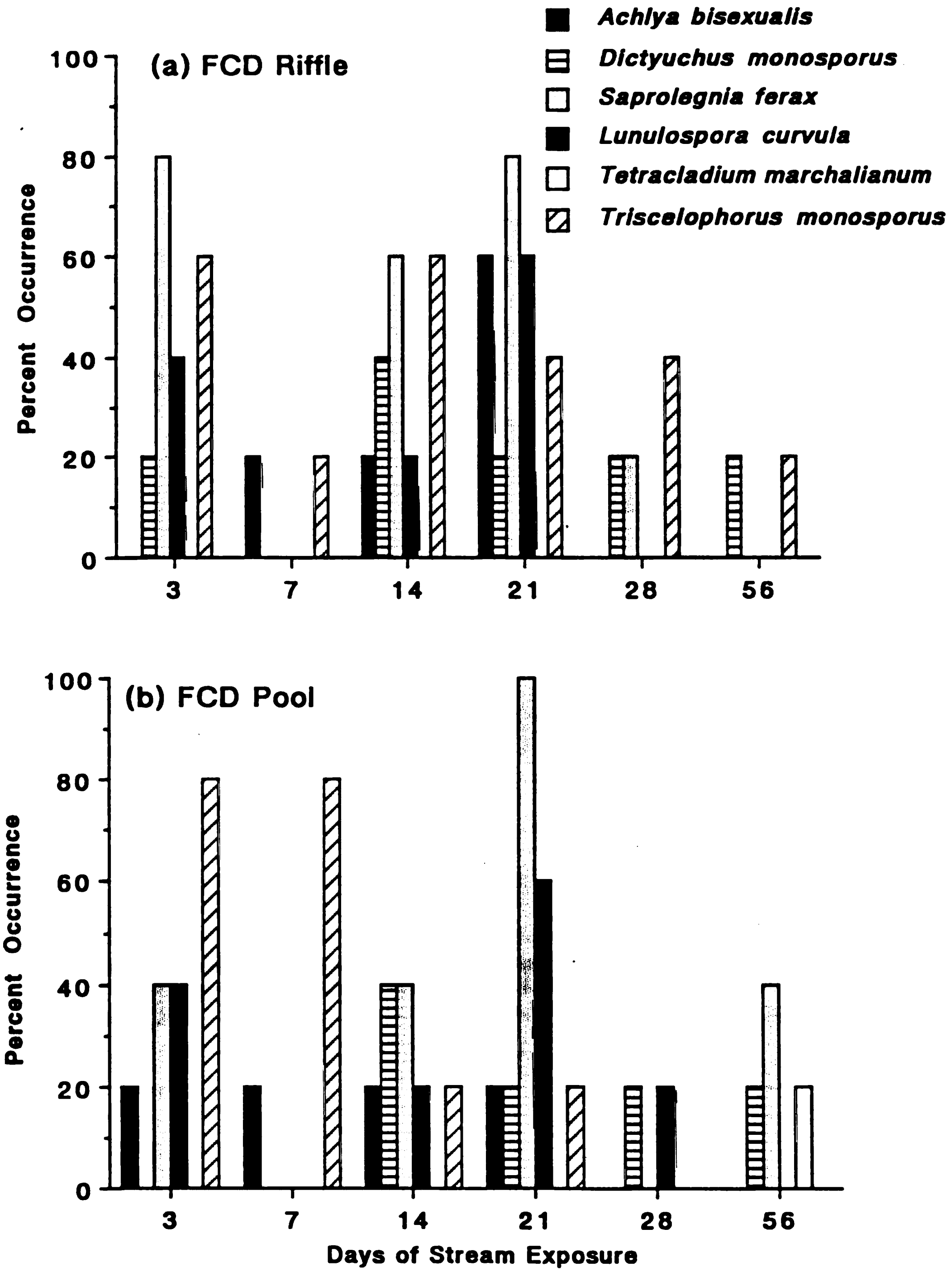


Figure 2 Percent Frequency of Occurrence of Select Species at FCD Riffle (a) and FCD Pool (b) sites from July 29 to September 10, 1990.

Table 6 Community similarity analyses between sites using Jaccard's Coefficient (CC_j) = $c/(S_1+S_2-c)$ and Sørensen's Coefficient (CC_s) = $2c/(S_1+S_2)$.

Comparison :		FEX-R vs FEX-P	FCD-R vs FCD-P	FEX-R vs FCD-R	FEX-P vs FCD-P
July 29 to September 10, 1990					
Hyphomycetes					
	S_1	6	5	6	5
	S_2	5	7	5	7
	c	5	5	5	5
	CC_j	0.83	0.71	0.83	0.71
	CC_s	0.91	0.83	0.91	0.83
Oomycetes					
	S_1	6	3	6	10
	S_2	10	6	3	6
	c	4	3	2	4
	CC_j	0.33	0.50	0.29	0.33
	CC_s	0.50	0.67	0.44	0.50
September 9 to December 10, 1990					
Hyphomycetes					
	S_1	8	10	8	10
	S_2	10	10	10	10
	c	7	8	7	8
	CC_j	0.64	0.67	0.64	0.67
	CC_s	0.78	0.80	0.78	0.80
Oomycetes					
	S_1	3	2	3	5
	S_2	5	4	2	4
	c	2	1	1	2
	CC_j	0.33	0.20	0.25	0.29
	CC_s	0.50	0.33	0.40	0.44

Table 7 List of species observed and total frequency of occurrence between July 12 to August 19, 1991 and August 16 to September 13, 1991 at FEX and FCD sites.

	7/12-8/19		8/16-9/13	
Species	FEX	FCD	FEX	FCD
Hyphomycetes				
Alatospora acuminata	33.30	38.89	28.93	19.93
Anguillospora crassa	0.00	0.00	4.40	0.00
Anguillospora longissima	11.11	15.67	28.80	24.40
Articulospora grandis	0.00	0.00	2.20	0.00
Articulospora tetracladia	1.83	1.83	4.40	0.00
Camposporium pellucidum	0.00	0.00	4.40	2.20
Centrospora acerina	1.83	1.83	0.00	2.20
Centrospora aquatica	1.83	3.67	0.00	6.67
Clavariopsis aquatica	9.17	11.06	11.07	6.67
Clavatospora tentacula	1.83	5.50	0.00	0.00
Flagellospora curvula	5.50	9.17	2.20	2.20
Flagellospora penicillioides	0.00	1.83	0.00	0.00
Lemonnierra aquatica	3.67	3.67	6.60	0.00
Lemonnierra terretris	0.00	3.67	0.00	0.00
Lunulospora curvula	59.22	63.89	48.93	46.67
Tetrachaetum elegans	0.00	7.44	0.00	0.00
Tetracladium marchalianum	57.39	57.44	71.07	86.67
Tetracladium setigerum	0.00	3.72	0.00	2.20
Tricladium angulatum	5.50	3.67	6.67	11.20
Tricladium giganteum	3.72	1.83	0.00	0.00
Triscelophorus monosporus	44.33	49.94	42.27	37.73
Oomycetes				
Achlya americana	7.33	11.06	0.00	0.00
Achlya bisexualis	5.50	23.11	11.00	13.27
Achlya colorata	0.00	0.00	2.20	0.00
Achlya hypogyna	0.00	1.83	0.00	0.00
Achlya polyandra	5.56	5.56	0.00	0.00
Achlya prolifera	0.00	0.00	2.20	0.00
Achlya proliferoides	1.83	0.00	2.20	0.00
Achlya racemosa	3.67	3.72	2.20	4.40
Dictyuchus monosporus	7.39	3.67	0.00	4.40
Saprolegnia australis	0.00	3.67	4.40	0.00
Saprolegnia diclina	8.28	4.61	2.20	6.60
Saprolegnia ferax	44.50	49.06	19.87	13.33
Saprolegnia glomerata	1.83	0.00	0.00	0.00
Saprolegnia hypogyna	1.83	0.00	0.00	0.00
Saprolegnia litoralis	7.39	1.83	2.20	0.00
Saprolegnia parasitica	5.50	8.28	6.60	6.60
Saprolegnia terrestris	1.83	0.00	0.00	0.00

bisexualis and *Saprolegnia ferax* are again the predominant species. However, several other oomycetes occur as frequently as *Dictyuchus monosporus* making it a less pronounced colonizer. There was a notable increase in the diversity of species seen during the summer of 1991 as compared to the summer of 1990.

Successional patterns for each of the selected species over the time during the summer 1991 studies do not suggest obvious patterns over the first 22 days of colonization (Figures 3 and 4). There is a suggestion that *Lunulospora curvula* is more important during early colonization than are other species (Figures 3 and 4). Comparisons of the mean frequency of occurrence of all species, hyphomycete species, oomycete species, and selected species resulted in significant correlations ($p < 0.05$) for each comparison (Table 8). Spearman correlations of the all species and the select species comparisons confirmed these results (Table 4). Paired t-tests revealed non-significant differences ($p < 0.05$) between sites for all comparisons except the all species and the hyphomycete comparisons of the July 12 to August 19, 1991 period. Jaccards's coefficient (CCj) and Sorensen's coefficient (CCs) of community similarity between FEX and FCD sites during both sampling periods resulted in coefficients that were greater for the hyphomycetes than the oomycetes (Table 9). Similarity between sites for the first sampling period was also greater than the second sampling period, possibly due to fewer species present during the second experiment. A community similarity comparison of hyphomycete and oomycete species between years using Jaccard's and Sørensen's equations gave coefficients for hyphomycetes of 0.50 and 0.67 and for oomycetes of 0.48 and 0.65.

For the July 12 to August 19, 1991 sampling period, the occurrence of the selected species as well as leaf weight remaining, was tested for correlation

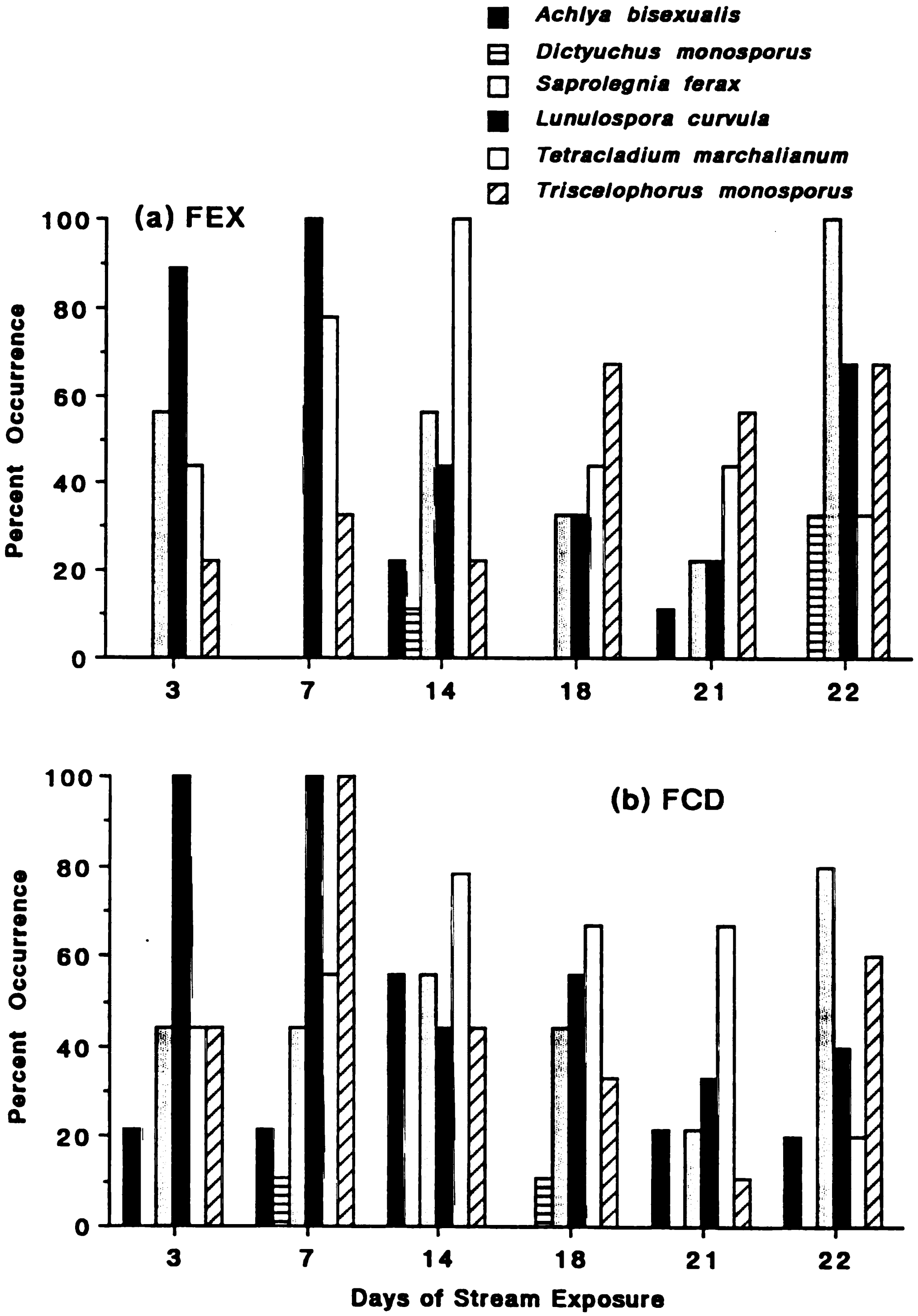


Figure 3 Percent Frequency of Occurrence of Select Species at FEX (a) and FCD (b) from July 12 to August 19, 1991.

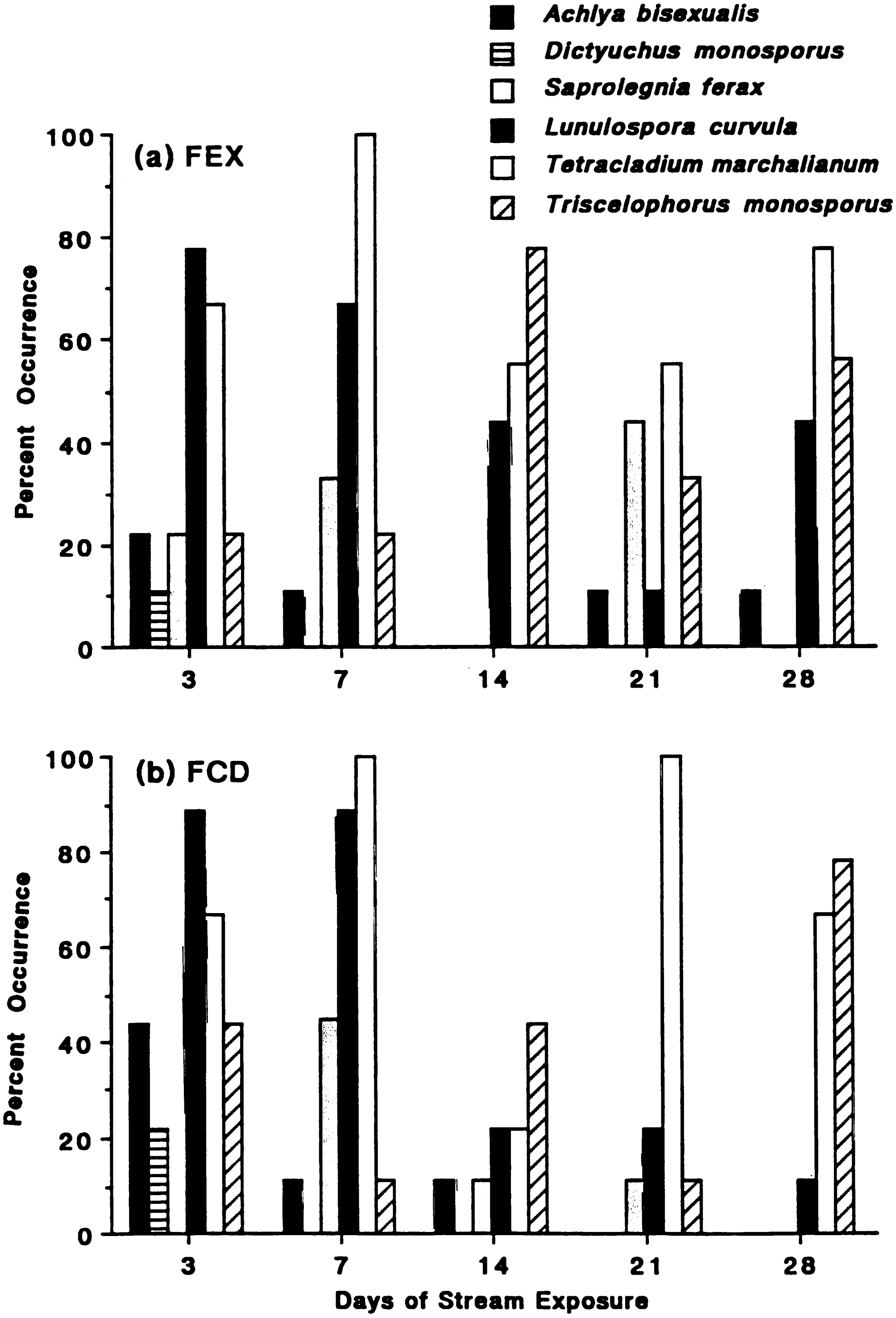


Figure 4 Percent Frequency of Occurrence of Select Species at FEX (a) and FCD (b) August 16 to September 13, 1991.

Table 8 Paired t-values and correlation coefficients for the frequency of occurrence of species between FEX and FCD sites for the summer 1991 sampling periods, N = number of species.

Comparison	N	t-value	probability	correlation coefficient
July 12 to August 19, 1991				
All Species	38	-2.388	0.022*	0.974**
Hyphomycetes	21	-3.697	0.001**	0.992**
Oomycetes	17	-0.653	0.523	0.910**
Selected Species	6	-1.629	0.164	0.954**
August 16 to September 13, 1991				
All Species	38	0.747	0.460	0.968**
Hyphomycetes	21	0.551	0.588	0.972**
Oomycetes	17	0.555	0.586	0.840**
Selected Species	6	-0.454	0.669	0.970**

* $p < 0.05$
** $p < 0.01$

Table 9 Community Similarity analyses between sites during the summer of 1991 and between years using Jaccard's Coefficient (CCj) and Sørensen's Coefficient (CCs).

	7/12/91 to 8/19/91		8/16/91 to 9/13/91	
	Hyphomycetes	Oomycetes	Hyphomycetes	Oomycetes
FEX vs. FCD				
No. of Species at FEX (S_1)	14	13	13	10
No. of Species at FCD (S_2)	18	11	12	6
No. of Species at both sites (c)	14	9	9	5
Jaccard's coefficient (CC_j)	0.77	0.60	0.56	0.45
Sorensen's coefficient (CC_s)	0.88	0.75	0.72	0.63
1990 vs. 1991				
No. of Species in 1990 (S_1)	12	14		
No. of Species in 1991 (S_2)	21	17		
No. of Species in both years (c)	11	10		
Jaccard's coefficient (CC_j)	0.50	0.43		
Sorensen's coefficient (CC_s)	0.67	0.65		

with water velocity and water temperature (Table 10). *D. monosporus* and *T. marchalianum* were negatively correlated with water velocity at FCD, while *S.ferax* was positively correlated. *L.curvula* was positively correlated with water velocity at FEX and with water temperature at FCD. However, since no consistent patterns were evident, these comparisons were not made for any of the other dates.

3. Leaf Decomposition Data

a. Summer 1990 - Leaf Area Loss Data

Percent leaf area remaining during the July 29 to September 10, 1990 stream incubation period indicate that loss of leaf area at pool sites was lower than at riffle sites (Figure 5). This may be a function of the near zero stream velocity in the pools that may have resulted in less physical abrasion. Decomposition rate (-k) calculated at each site also indicate faster rates of decomposition at the riffle sites (FEX = 0.00304 and FCD = 0.00531) than at their respective pool sites (FEX = 0.00180 and FCD = 0.00284). These rates would be considered slow based on the classification of Peterson and Cummins (1974). By the end of the 42 day period, the sites had only lost 23, 34, 18, and 25% of the original leaf area. This could be a reflection of the sampling method employed. The bricks to which the leaves were attached may have provided support and protection against the stream current. Also, the tough outer leaf matrix of the fresh leaf may have inhibited microbial colonization to some degree. However, the leaves had disappeared by October 29. An increase in the amount of leaf input in the stream and an associated increase in the diversity of aquatic hyphomycete spores, as is evident from the early autumn 1990 sampling period, and possibly numbers of fungal spores probably

Table 10 Correlation coefficients for water velocity and temperature vs. species frequency of occurrence from July 12 to August 19, 1991 at FEX and FCD sites.

Comparison	FEX	FCD
Water Velocity vs Species Frequency		
Achlya bisexualis	-0.238	-0.045
Dictyuchus monosporus	0.648	-0.741*
Saprolegnia ferax	0.672	0.861*
Lunulospora curvula	0.716	0.451
Tetracladium marchalianum	-0.023	-0.805*
Triscelophorus monosporus	-0.305	0.647
Water Temperature vs Species Frequency		
Achlya bisexualis	-0.615	-0.419
Dictyuchus monosporus	-0.625	0.061
Saprolegnia ferax	-0.658	-0.430
Lunulospora curvula	0.403	0.764*
Tetracladium marchalianum	-0.311	-0.297
Triscelophorus monosporus	-0.038	0.447
Water Velocity vs Leaf Weight Remaining	-0.277	0.001
Water Temp vs Leaf Weight Remaining	0.591	0.588

* $p < 0.05$

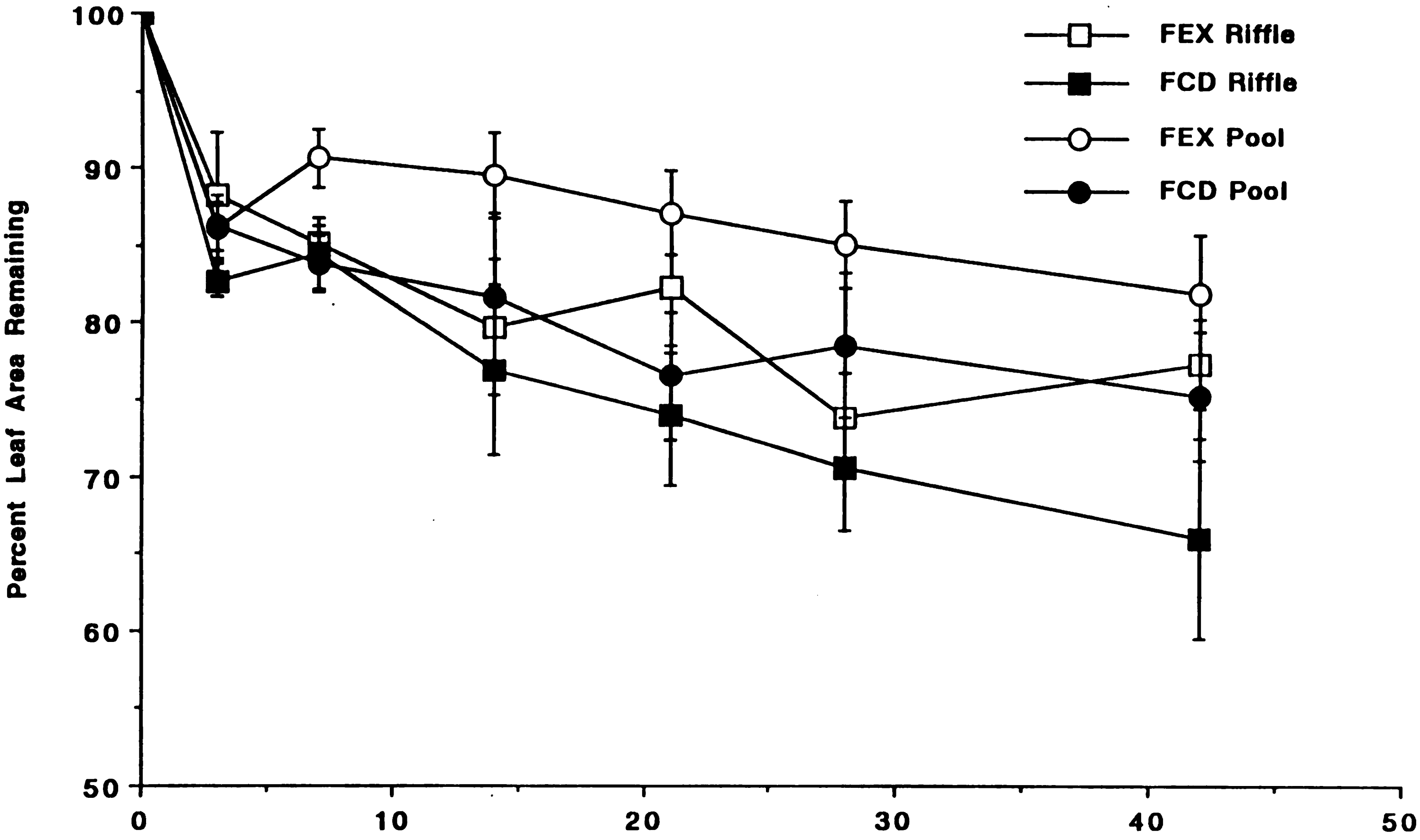


Figure 5 Percent Leaf Area Remaining from July 29 to September 10, 1990.
Error bars = +/- 1 S.E.

t
a
C
a
th
er
ma
to
cor

contributed to the decomposition of the leaves (Barlocher and Kendrick 1974, Shearer and Webster 1985b).

A two-factor ANOVA of the percent leaf area remaining at FEX riffle, FEX pool, FCD riffle and FCD pool was performed for the summer of 1990 sampling period, with site and date as the grouping factors (Table 11). Specific comparisons included all sites, FEX riffle versus FCD riffle, FEX pool versus FCD pool, FEX riffle versus FEX pool, and FCD riffle versus FCD pool. Significant site and date effects were apparent, however, there were no significant interaction effects. Paired t-tests and correlations on sqrt*arcsin transformed data indicated significant differences between sites ($p < 0.05$) but a high degree of correlation ($p < 0.01$ or $p < 0.05$) (Table 12).

b. Summer 1991 - Leaf Weight Loss Data

Percent leaf weight remaining throughout the sampling periods from July 12 to August 19, 1991 and August 16 to September 13, 1991 indicated that decomposition rates during both periods were faster than the previous year (Figure 6). The rate of leaf decomposition (-k) at the FCD site was slightly faster than that at the FEX site during both the July 12 to August 19 (FCD = 0.0423 and FEX = 0.0399) and the July 16 to September 13 (FCD = 0.0283 and FEX = 0.0212) sampling periods. These rates would be considered fast by Peterson and Cummins (1974). These rates are faster than those found in 1990 using the same leaf species, probably because of differences in the sampling method employed. Suspension of the leaves in the water by their petioles appears to make them more susceptible to fragmentation by stream currents and possibly to decomposition by microorganisms due to a greater surface area available for colonization. Attachment of leaves to bricks is probably a better method to use

Table 11 Two-Factor ANOVA of the percent of leaf remaining during the 1990 and 1991 sampling periods.

Date	Source	Site (A)	Date (B)	AB
July 29 to Sept 10, 1990				
	All Sites	9.706*	6.424*	0.687
	Riffle Sites	6.489*	5.088*	0.587
	Pool Sites	10.790*	1.945	0.550
	FEX Sites	9.812*	2.747*	1.170
	FCD Sites	4.172*	3.875*	0.452
July 12 to Aug 19, 1991				
	FEX vs FCD	4.416*	19.869*	1.166
Aug 16 to Sept 13, 1991				
	FEX vs FCD	0.169	29.791*	0.740

* significant at the $p \leq 0.05$ level

Table 12 Paired t-tests and correlations of sqrt*arcsin transformed percent leaf remaining data for the summers of 1990 and 1991.

Comparison	Paired t-value	Probability	Correlation Coefficient
Summer 1990			
FEX Riffle vs FEX Pool	-2.641	0.0385*	0.831*
FCD Riffle vs FCD Pool	2.807	0.0309*	0.871**
FEX Riffle vs FCD Riffle	3.588	0.0115*	0.801*
FEX Pool vs FCD Pool	-2.662	0.0374*	0.919**
Summer 1991			
FEX vs FCD, 7/12-8/19	2.203	0.0635	0.943**
FEX vs FCD, 8/16-9/13	0.184	0.8613	0.938**

* $p < 0.05$
** $p < 0.01$

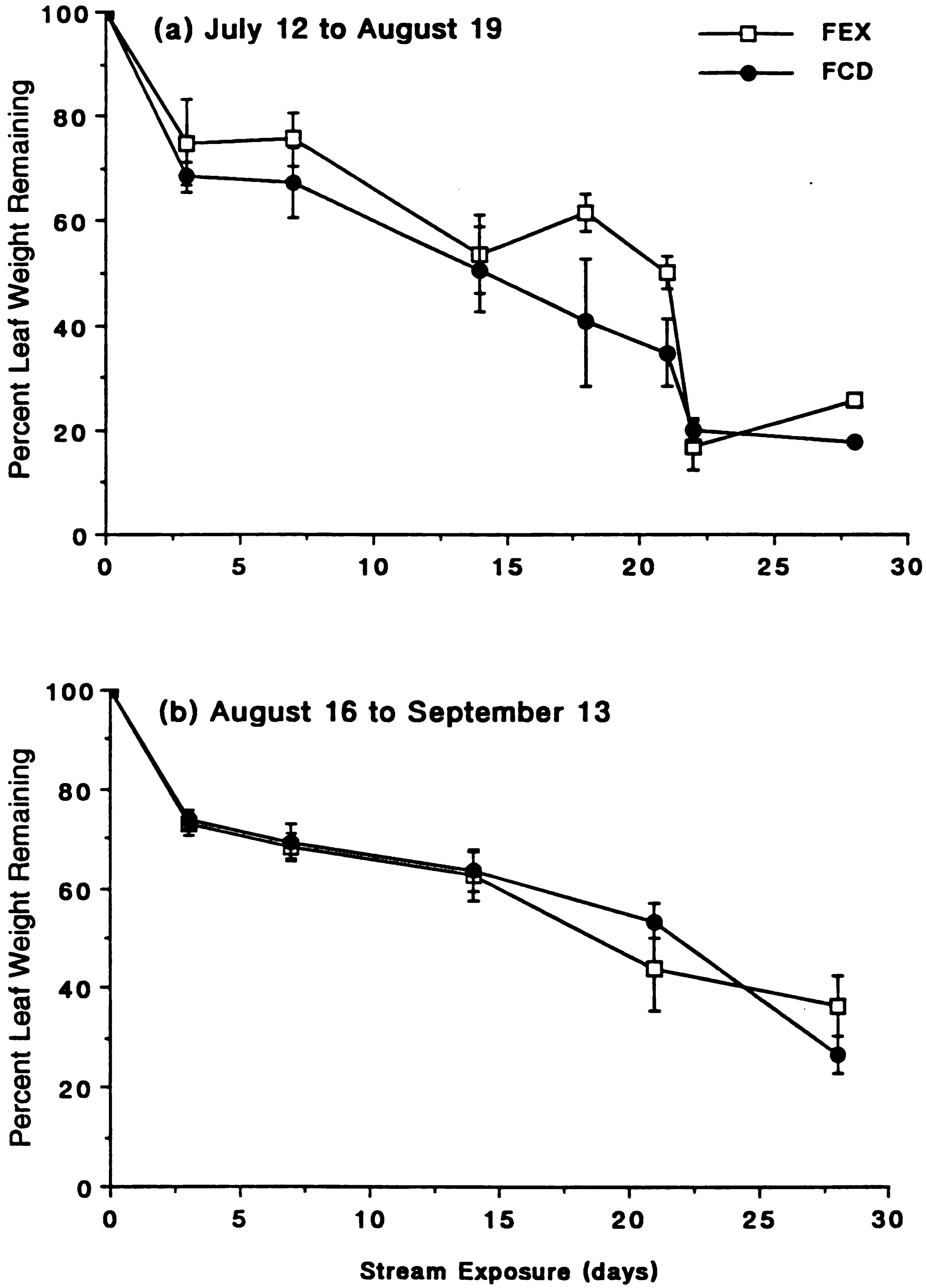


Figure 6 Percent Leaf Weight Remaining at FEX and FCD Sites during the 1991 Summer Sampling Periods. Error bars = +/- 1 S.E.

to describe the system since more often leaves are found aggregated against rock or wood surfaces within streams.

A two-way ANOVA of the percent leaf weight remaining at FEX and FCD sites, with site and date as the grouping factors resulted in significant site and date effects during the July 12 to August 19 sampling but a non-significant interaction effect (Table 11). A similar ANOVA for the July 16 to September 13 sampling period resulted in a significant date effect but non-significant site and interaction effects. Paired t-tests and correlation coefficients of sqrt*arcsin transformed data indicate that the sites are not significantly different ($p > 0.05$) and are highly correlated ($p < 0.01$) during both sampling periods (Table 12).

4. Nutrient/Chemical/Physical Parameters at Study Sites

Sampling was conducted during the hours of 10:00 to 14:00 to insure consistency of the results. Velocity data calculated at each brick for the summer of 1990 and at the front and the back of the sampling box used for the summer of 1991 indicated that velocity was usually slightly higher at FCD than at FEX (Figure 7) in 1990, while the reverse was true in 1991 (Figure 8). Only the riffle sites for 1990 are shown due to a velocity of zero at the pool sites on nearly every sampling date. Water temperature ranged from 16°C to 23°C during the summer of 1990 and from 13°C to 25.5°C during the summer of 1991, and there were no consistent differences between the two sites (Figure 7 and 8).

Average concentrations of ammonia, chloride, nitrate, nitrite and soluble reactive phosphorus and a number of physical parameters were calculated for the following sampling periods: July 29 to September 10, 1990, September 9 to October 29, 1990, October 29 to December 10, 1990, July 12 to August 19, 1991, and August 16 to September 13, 1991 and are given in the appendix (Tables A-9 and A-10). Nutrient, physical and chemical values were consistent

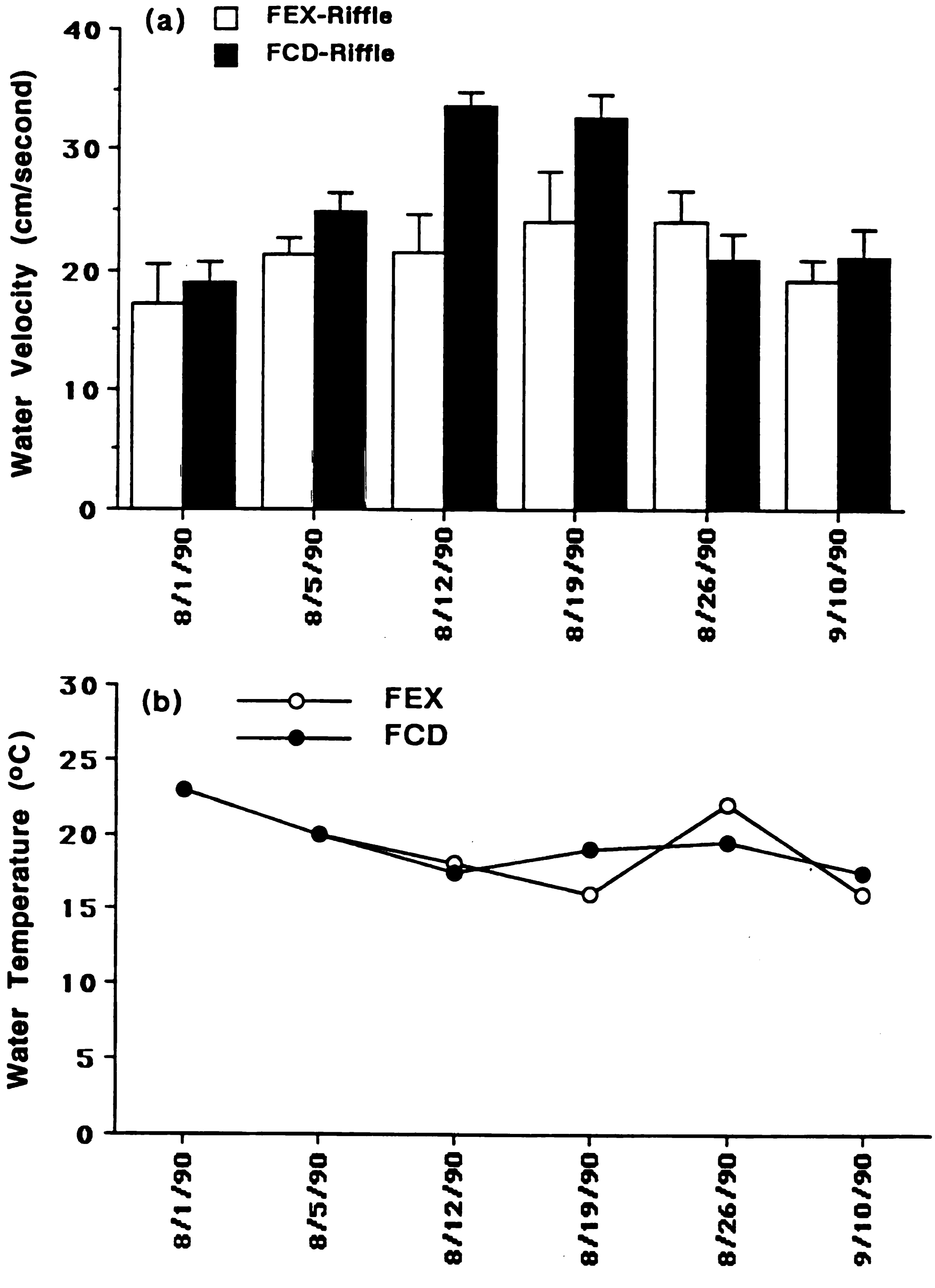
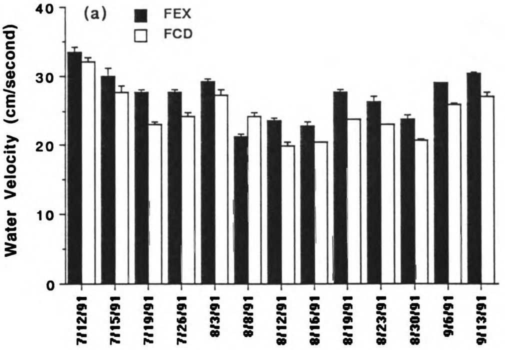


Figure 7 Stream Velocity at Riffle sites (a) and Water Temperature (b) at FEX and FCD during the Summer of 1990. Error bars = +/- 1 S.E.



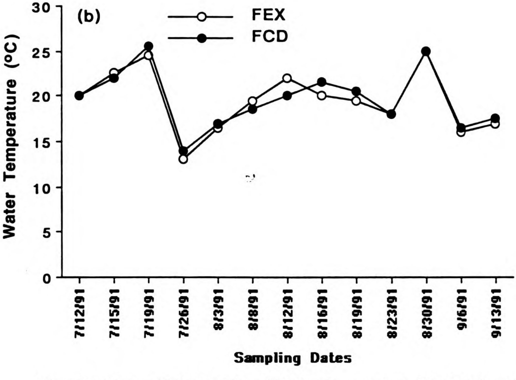


Figure 8 Stream Velocity (a) and Water Temperature (b) at FEX and FCD during the Summer of 1991. Error bars = +/- 1 S.E.

between sites and similar for the summers of 1990 and 1991 with increased concentrations of nutrients, especially ammonia and nitrate, and a general decrease of the physical parameters during the fall and winter of 1990.

DISCUSSION

Aquatic hyphomycetes and aquatic oomycetes were found in riffle, run and pool habitats in this study. This study suggests that oomycetes are as widely distributed int he stream as the more widely studied hyphomycetes and warrent more attention than they have received heretofore. The aquatic hyphomycetes were first noticed in small lakes and ponds by deWildeman in the late 1800's and were first discovered in streams by Ingold (1942). Research on the aquatic hyphomycetes has centered on their existence in the faster flowing water since the 1940's. Nilsson (1964) noted higher species diversity and greater fungal biomass on leaves in well-aerated streams and Webster and Towfik (1972) claimed that turbulence was required for the sporulation of some aquatic hyphomycetes. It was suggested that the material settling in pools may be subject to anaerobic conditions unsuitable for the germination and growth of most hyphomycete spores due to sedimentation. Aquatic oomycetes may be more tolerant of conditions in the pools, since they are more tolerant of low oxygen conditions. Thus, they may benefit from the incapacitation of aquatic hyphomycetes in pools allowing them to proliferate even in the presence of organisms that under normal conditions would outcompete them for food and/or space.

Whether the spores of either the oomycetes or the hyphomycetes were actively germinating or attached in a dormant state until ideal conditions were present was not explored in this study. Species abundance measures allow one to speculate on the importance of individual species relative to the other members of the community. However, in order to address the importance of a species during the decomposition of leaves within the stream it would be helpful to obtain an estimate of biomass on the leaves, rather than just the occurrence of spores, and also to determine the function of a species during decomposition by measuring the activity of enzymes that degrade leaf components.

There was no significant difference in the diversity of fungal species in the various habitats for either fungal class. However, there appeared to be a greater site to site difference when the frequency of species within each class was compared. The community similarity was always quite low for the oomycetes, especially during the 1990 field season. This suggests that the species composition within the oomycete class was highly variable. Not only were different sites along the river dissimilar but the community makeup within riffle and pool subsites also varied. Roberts (1963) attributes this sporadic isolation to the inability of some species to swim far before encysting. Thus, oomycetes are poor dispersers and are not well distributed.

This study confirmed the observation that the aquatic hyphomycete community on a leaf is dominated by a small core group of species that arrive at the onset and persist throughout the life of the leaf while rarer fungal species occur sporadically. There was no consistent succession of species on leaf surfaces. Results also agree with a study by Chamier and Dixon (1982a) that concluded that species succession was not apparent on alder leaf packs. There were approximately four species of hyphomycetes present continuously during both field seasons. During 1990 the hyphomycetes *Lunulospora curvula, Triscelophorus monosporus, Tetracladium marchalianum* and *Articulospora tetracladia* were constant members of the community and in 1991 *L. curvula, T. monosporus, T. marchalianum,* and *Alatospora acuminata* made up the core group of hyphomycetes. Some species, such as *Lunulospora* *curvula* and *A. acuminata,* peaked early during the decomposition process than steadily declined as substrate was diminishing while other species showed no definite pattern of peak and decline.

The issue of whether a core group of oomycetes persists throughout leaf degradation with random colonization by rarer species, as has been suggested for the hyphomycetes, or whether there is a succession of species that colonize at particular points during the decomposition process has not been adequately studied. *Achlya bisexualis, Saprolegnia ferax,* and *Dictyuchus monosporus* made up what might be interpreted as a core group of oomycetes in the Ford River in 1990 as did *A. bisexualis* and *S. ferax* and to a lesser extent *D. monosporus, S. diclina*, and *S. parasitica* in 1991. However, the pattern was not as distinct as for the hyphomycetes, perhaps as a result of their poor dispersal ability, as was mentioned previously. Roberts (1963) noted a succession of species colonizing hempseeds suspended in water, but this study is the first report of succession on leaves.

More research also needs to focus on the interaction between the various fungal communities on leaves to determine the extent to which one is affected by the other. It is possible that hyphomycetes outcompete the oomycetes for space due to their ability to release enzymes that initiate leaf decomposition which oomycetes may not possess, faster growth rate, or the release of compounds that inhibit oomycete production. In this study, it was apparent that the occurrence and diversity of oomycete species declined as hyphomycete diversity increased during the fall of 1990. However, this pattern may have been a result of dropping water temperature rather than competition. In order to fully understand the interaction between these two groups, an intense study of spore production, species diversity, and growth as influenced by biotic and abiotic factors, is necessary.

There was a pronounced temporal pattern noted for hyphomycetes during the 1990 field season. The seasonal response of the aquatic hyphomycetes seemed to be associated with the influx of detrital material early in the fall season. There was a marked increase in the diversity of fungi probably due to a decrease in competition for substrate. *Tetracladium marchalianum,* a minor component of the fungal community during the 1990 summer field season, became a major species during the fall of that same year. At the same time the frequency of summer species, such as *Lunulospora curvula* and *Triscelophorous monosporus*, began to decline as the water temperature dropped. This agrees with several authors (Ingold 1979, Sridhar and Kaveriappa 1989, Thomas *et al* 1989) who have associated these species with warm temperature in the tropics.

The temporal pattern noted within the oomycete class was slightly different. There appeared to be more species during the summer field season and a marked decline in the number of species with the onset of autumn. However, rarer species such as *Saprolegnia parasitica* were apparently more inclined to colonize leaves during the colder period and may be profitting from the decline in competition for substrate. These observations are contrary to numerous authors claiming the greatest diversity of species to occur during the winter (Coker 1923, Forbes 1935, Waterhouse 1942, Perrott 1960, Hughes 1962, Roberts 1963, Gupta and Mehrotra 1989). Only a handful of researchers have observed the highest number of species during the summer months (Khulbe and Bhargava 1977, Mer *et al* 1980).

As Suberkropp and Klug (1976) pointed out, the assemblage of organisms observed is affected by the mycoflora of the particular stream as well as the methods used for sampling and identifying species. In this study the leaf species used was *Alnus rugosa* during both field seasons. This species lined the stream and represented approximately 75% of the leaf material entering the stream, either as fresh leaves during the summer months or as senescent leaves during the autumn months. Fresh leaves were used during the 1990 field season and leached, dried fresh leaves during 1991. Barlocher (1990) suggests there is an inhibition of fungal growth on fresh versus dried leaves due to the retention of soluble substances such as phenolics, carbohydrates, and amino acids. The occurrence of *Tetracladium marchalianum* in the present study was notably different between years. This species was a late colonizer and minor component of the fungal community in 1990 but an early and prominent colonizer in 1991. It is possible that this species was inhibited by substances retained in the fresh leaves or does not possess the enzymes capable of degrading the tough matrix of an unleached leaf.

Research focusing on aquatic hyphomycete colonization led Barlocher (1992) to conclude that the absence of leaching in fresh leaves entering the stream results not only in a decrease in the number of species that initially colonize the leaf but a decrease in the productivity (no. of spores produced) of a given species. This may, in part, explain the increase in the number of species seen during the summer of 1991 as compared to the summer of 1990. Also, the slower degradation of the fresh leaves coincides with Gessner and Schwoerbels (1989) observation that there is no initial loss of weight of fresh alder and willow leaves due to leaching. Barlocher (1992) also suggests that oomycetes may profit from the retention of soluble substances in fresh leaves. However, there was no evidence in the present study that this was the case. In fact, the number of oomycete species also increased during the second field season.

Overall community similarity was fairly low between years and was most likely a reflection of the different sampling methods employed. During the first field season green leaves were attached to bricks such that only the top leaf surface was exposed for colonization. In the summer of 1991 dry leaves were suspended in the water column such that both the top and bottom surfaces were free to be colonized. Decomposition of the leaf substrate occurred more rapidly when the leaves were suspended thereby increasing exposure to the physical forces of the stream. One might expect that fewer species would be apparent due to the shortened residence time of the leaf and decreased likelihood of rarer or late-colonizing species. However, more oomycete and hyphomycete species were identified during the second field season. For both classes of fungi, multiple leaf discs on individual leaves were analyzed for spores during the second field season, as opposed to one disc per leaf during 1990. This may have been a more efficient method of determining the communities colonizing a particular leaf. Year to year variation, use of predried leaves and greater surface area potentially available to fungal colonization may also have been contributing factors to the greater number of species observed during 1991. Little research has focused on the difference in the accessibility or permeability of the two different surfaces of the leaf. Different species may preferentially colonize one surface over the other.

The implementation of different sampling techniques during the two years of sampling makes it difficult, if not impossible, to compare these two communities from year to year. Whether dried leaves or fresh leaves are more appropriate for characterizing fungi in streams depends on the season. While more species may colonize dried leaves during the summer months, the use of fresh leaves may provide a more realistic picture of the fungal activity since it is a more significant input during this season. Conversely, dried leaves are the major input during the fall and thus would be the more sensible sampling substrate. By using leaves as the sampling substrate, one can only speculate on the fungal leaf community and cannot extrapolate to the water column or scum fungal communities and assume that all species have been identified. Iqbal and Webster (1973) showed that the aquatic hyphomycete spore content of foam and river water were significantly different and sampling of one would provide only part of the information on the stream fungal community. The same is true for leaves. It is merely an estimate of the diversity of the stream community. The sampling method(s) chosen will depend on the question asked. This study emphasizes the need for multiple sampling methods, investigation of the various habitats within the stream, sampling during different seasons and long-term sampling.

CHAPTER 2 : Enzyme Study

INTRODUCTION

Aquatic fungal communities play numerous roles and warrant attention as important, if not vital, components of stream ecosystems. Research on aquatic hyphomycetes has provided evidence of their importance in stream dynamics through studies of leaf processing, extracellular enzyme production, succession as influenced by factors such as temperature, pH and substrate availability, and interactions with bacteria and invertebrates. Aquatic fungi increase the surface area of the detritus for bacterial colonization, break through the tough outer matrix that might be impenetrable to other organisms, and are also a food source for invertebrates (Suberkropp and Klug 1980). Bacteria and invertebrates lack the enzymatic ability to initiate the decomposition process and thus depend on the array of enzymes produced by the aquatic fungal community.

Leaf maceration by these organisms is a primary link between detritus and other heterotrophs within the stream ecosystem (Kendrick and Hynes 1971, Barlocher and Kendrick 1974, Suberkropp and Klug 1980). Production of enzymes that metabolize typical structural components of plants, such as cellulose, hemicellulose and pectin, are paramount to successful degradation of leaves. The aquatic hyphomycetes have been shown by several authors (e.g. Suberkropp and Klug 1980, Chamier and Dixon 1983, Suberkropp et al 1983, Chamier 1985) to be capable of producing the necessary enzymes.

However, similar studies of the aquatic oomycetes are lacking. The oomycetes have often been regarded as 'sugar' fungi unable to produce enzymes capable of breaking down the leaf material in the initial stages of decomposition (Thompstone and Dix 1985). Within the past 20 years, however, more and more evidence suggests that they have the ability to produce enzymes involved in the degradation process. Several genera of the aquatic oomycete community have been implicated as causal agents of invertebrate and vertebrate disease (Butty et al 1989, Hatai et al 1990). However, the fundamental role of other members of the aquatic oomycete class within the stream environment has not been well established, in particular their contribution to detrital decomposition. Several oomycete species can be isolated from leaves and twigs upon baiting and uninduced growth on these substrates has been observed (Chapter 1, Rogers, personal communication). A physiological examination of species within this class, focusing on detrital decomposition, may provide information regarding the role of this class of fungi during decomposition of plant organic matter.

A stream survey conducted during the summers of 1990 and 1991 showed that *Lunulospora curvula* Ingold, *Tetracladium marchalianum* de Wildemann and *Triscelophorus monosporus* Ingold were among the more predominant members of the aquatic hyphomycete community isolated from leaves from the Ford River in Northern Michigan. Those results agreed with other studies of temperate streams (Ingold 1979, Chamier and Dixon 1985, Chergui and Pattee 1988, Suberkropp 1991). These species were selected for an *in vitro* study to determine if their abundance in the stream ecosystem was related to an ability to decompose organic matter, particularly leaves. The oomycete species *Achlya bisexualis* Coker and Couch, *Saprolegnia ferax* (Gruith) Thuret and *Dictyuchus monosporus* Leitgeb were also frequently isolated from leaves from the Ford River and were selected on this basis in an attempt to determine their role in decomposition of leaf material relative to the selected members of the hyphomycete community. The objective of this study was to compare the enzymes produced by the predominant species of hyphomycetes and oomycetes colonizing leaf material in the Ford River to help to clarify the relationship between these two classes of fungi within the aquatic environment. The hypothesis to be tested was that oomycetes are capable of producing the same enzymes for catalysis of leaf cell wall components as are hyphomycetes and, thus, are important, but mostly overlooked, decomposers of leaf detritus in streams.

LITERATURE REVIEW

Suberkropp et al (1976) documented changes in the chemical composition of oak and hickory leaves as decomposition progressed. They discovered that different components varied significantly in their relative importance over time. Soluble compounds such as reducing sugars and polyphenols lost most of their weight within the first 24 hours of exposure due to leaching. Cellulose and hemicellulose components lost weight more gradually over time but the relative percentage of the total leaf remained the same. Lipids not only lost weight over time but the relative percentage also decreased. Both nitrogen and lignin increased in weight during the first several weeks as well as percentage of the total leaf.

According to Darvill (1980), pectic polysaccharides make up 34% of the primary cell wall of a plant and cellulose and hemicellulose 24% and 18% respectively. In order for organisms to exploit leaf components they need to be able to produce cellulase, hemicellulase and pectinase enzymes. Different modes of action exist for the various enzymes. Some cleave the terminal or subterminal bonds (exo-enzymes) while others randomly cleave along the polymer chain (endo-enzymes). Often enzymes must act in conjunction with one another in order to fully degrade a native substrate. The cellulases endocellulase, exocellulase and cellobiase are thought to act in a synergistic manner during the degradation of cellulose (Wood and McCrae 1972). During decomposition cellulose is hydrolyzed into glucose and soluble celloligosaccharides. Endocellulases randomly hydrolyze ß-1,4 glycosyl bonds in the interior of the cellulose polymer while exocellulases (e.g. cellobiohydrolase, ß-glucosidase) hydrolyze the bonds near the non-reducing ends of the polymer (Reese 1977, Eriksson 1978).

Microorganisms have been shown to produce enzymes capable of degrading pectin which leads to the decomposition of living and dead plant tissue (Bateman and Millar 1966, Bateman and Basham 1976, Torzilli 1978). Aquatic hyphomycetes were found to dominate the microbial community at the onset of leaf decomposition (Triska 1970, Kaushik and Hynes 1971, Barlocher and Kendrick 1974, Suberkropp and Klug 1976) and were proposed to control this important function in the stream environment. Researchers suspected the role of these organisms in decomposition not only had important implications for the success of the fungal species themselves but also for the numerous other micro- and macro-organisms within the foodweb (Suberkropp and Klug 1980). Golladay and Sinsabaugh (1991) concluded from structural and functional studies of biofilm development on leaves and wood that fungi were an important part of the biofilm but were limited on leaves due to the physical instability and ephemeral nature of this substrate. Leaf ergosterol, a measure of viable fungal biomass, peaked early as did several enzymes implicated in decomposition.

Early studies showed aquatic hyphomycetes had an ability to grow on cellulosic substrates (Ranzoni 1951, Tubaki 1958, Nilsson 1964) and also exhibited weak pectinase activity on a pectin substrate (Tubaki 1958). Research began focusing on the enzymes elaborated by the aquatic hyphomycetes during leaf decomposition (Suberkropp and Klug 1976, 1980, Suberkropp et al 1983). Pure culture studies of individual fungal species showed that different species had varying degrees of enzyme production but many were able to degrade cellulose, pectin and xylan (Suberkropp and Klug 1980). Barlocher and Kendrick (1974) showed there was a synergistic effect on leaf decomposition when fungi were inoculated simultaneously. The elaboration of some enzymes for the degradation of certain plant components seems to depend on the chemical environment. Suberkropp and Klug (1980) identified pectin lyase as one of the initial enzymes excreted by hyphomycetes. It is a key enzyme in leaf maceration since pectic polysaccharides are the major components of plant cell walls. They suggested that the concentration of calcium was an important factor in the production of pectin lyase. Chamier and Dixon (1983) also found that increasing the calcium ion concentration led to an increase in the pectin lyase activity. Chamier (1985) substantiated this finding in a study that showed polygalacturonase, cellulase and hemicellulase activity by aquatic hyphomycetes to be greatest under acidic conditions whereas pectin lyase activity showed optimum values in alkaline conditions.

Chamier (1987) compared rates of decomposition and microbial colonization in streams that varied in pH from 6.8 to 4.9. She found that regardless of the type of vegetation, decay rates and levels of colonization were higher at pH 6.8. However, the magnitude of the difference was associated with leaf type. Thompson and Barlocher (1989) found that weight loss of maple and oak leaves increased with pH and temperature. They noted that in a laboratory with constant temperature conditions and no invertebrates, decomposition due to microbial activity peaked at 5.5 to 6.0. However, peaks of decomposition activity were species-specific when sterile leaves were inoculated with individual species of aquatic hyphomycetes and corresponded loosely to the natural occurrence of the species in acid or alkaline streams.

Bengtsson (1983) showed habitat selection in two aquatic hyphomycete species. Chamier (1985) suggested that the success of an aquatic hyphomycete species on alder leave was due to differences in enzymatic responses and was correlated with its ability to utilize cellulose. Thomas et al

(1992) surmized that along with enzymatic abilities, differences in the concentrations of fungal nutrients contributed to substrate preferences. Several researchers have concluded that levels of enzyme activity by aquatic hyphomycete species were not correlated to fungal growth, leaf weight loss or softening of leaves (Suberkropp et al 1983, Butler 1984, Butler and Suberkropp 1986).

Leaves as a food source for stream detritivores is enhanced by fungal colonization and conditioning (Barlocher and Kendrick 1973, 1975, 1981, MacKay and Kaff 1973, Kostalos and Seymour 1976). Preference of individual fungal species on selection and growth by stream invertebrates such as amphipods (Barlocher and Kendrick 1973) and caddisflies (Suberkropp et al 1983) has been demonstrated and has been correlated with growth of these invertebrates. Researchers concluded that invertebrates select leaves based on the degradative capacity of a leaf, the extent of fungal colonization and growth, and the production of enzymes by fungi. Chandrashekar et al (1989) explored the affect of aquatic hyphomycete colonization on the feeding preference of a detritivorous fish and discovered a correlation between the leaf palatabilty, duration of fungal colonization and protein content.

Unestam (1966) was one of the first researchers to examine the ability of saprophytic fungi, including a species of *Saprolegnia*, to produce enzymes involved in degradation. He discovered that the saprophytic species in the study showed no cellulolytic activity toward ground filter paper using a turbidometric method and very little pectinolytic activity, viscometrically measured. However, other researchers found some aquatic oomycete species were able to liberate reducing sugars from cellulose substrates. Berner and Chapman (1977) studied the celluloytic activity of six oomycetes. Since some showed an ability to hydrolyze cellulose substrates, they concluded that more research focusing on this group of fungi was warranted . As Thompstone and Dix (1985) pointed out, the term "sugar fungi" should not be applied freely to the oomycete class. While the ability of fungi to degrade cellulose varies, they reported 27 isolates of *Achlya* and *Saprolegnia* spp. that produced the endo-glucanase (i.e. endocellulase) enzyme which released reducing sugars from carboxymethylcellulose. They suggested that members of the Saprolegniaceae family may contribute more to the recycling of carbon from cellulose than previously thought, especially since celluloses are swollen in aquatic environments and have a lower crystalline content than dry plant material resulting in an increased accessibility. Many oomycetes can utilize amino acids as a source of carbon and energy (Faro 1971, Gleason 1968, Gleason et al 1970a, b).

Mitchell and Deacon (1986) studied the behavioral responses of a few oomycete species and determined that while the initial contact of zoospores on chitin or cellulose substrate occurred at random it was followed by a behavioral change that implied some sort of surface recognition. They suggested that zoospores of these fungi might be able to select suitable substrates in nature for successful encystment and growth.

Interest in the aquatic oomycete *Achlya bisexualis,* due to its unique mode of reproduction, resulted in the furthering of knowledge regarding species within the oomycete class. Research indicated that the cell wall of *A. bisexualis* was 15 to 60 % cellulose (Parker et al 1963, Novdes-Ledieu et al 1967). It was postulated that cellulolytic activity was important for the growth of this organism (Thomas and Mullins 1967, 1969). The hypothesis was that cellulase softens the fungal walls, allows change in the wall structure, and then is secreted. They observed that wall extension and branching increased simultaneously with an increase in cellulase levels. Interest by Miele and Linkins (1978) revealed the ability of *A. bisexualis* to degrade carboxymethylcellulose and microcrystallinecellulose substrates for uptake and nutrition via the adsorption of cellulase to the substrates and cellulose component.

METHODS AND MATERIALS

1. Growth Study

The particular species of hyphomycetes and oomycetes were chosen based upon field stream frequency studies described in the previous chapter. *Lunulospora curvula, Tetracladium marchalianum* and *Triscelophorus monosporus* were the aquatic hyphomycetes seen most frequently on leaves attached to bricks and submerged in the Ford River during the summer of 1990. Leaf discs were cut from each of five randomly selected leaves, aerated and the spores examined and identified under a light microscope every two days (Ingold 1975, Barlocher 1982). The species were isolated in pure culture on water agar plates containing chloramphenicol to prevent bacterial contamination. This simple medium was used to provide an attachment site for the spores which did not promote rapid growth of the individual spores or possible contaminants in order to allow isolation of the individual conidia (M.J. Klug, personal communication). Hyphomycete conidia were isolated by pipetting drops of the distilled water used during aeration onto the water agar plates, allowing several hours for germination , and cutting out the area of agar surrounding a germinating spore and transferring to an agar plate containing a mineral salts medium (see Appendix Two).

The oomycetes sampled in the Ford River belonged to the Saprolegniales order. The species observed most frequently included *Saprolegnia ferax, Achlya bisexualis,* and *Dictyuchus monosporus.* Leaves were sampled as for the hyphomycetes. However, leaf discs were incubated in a petri dish containing distilled water and several sterilized half hempseeds to promote colonization and growth of the spores (Coker and Matthews 1937, Johnson 1956, Seymour 1970). Hempseeds are most often employed in the baiting of genera from the Saprolegniales because they provide a concentrated nutrient substrate and the ability to sustain organisms for long periods of time. The sterilization of the hempseeds diminishes the possible contamination by bacteria. The oomycetes were isolated by cutting hyphal tips from the colony growing in the petri dish and transferring them to water agar plates with chloramphenicol.

Isolates of oomycetes and hyphomycetes were then transferred from the water agar plates to mineral salts plus 1 % glucose agar plates and Yeast-Peptone-Glucose (YpG) agar plates. These media were selected to provide the nutritional requirements needed to sustain and promote growth of the organisms (Barlocher and Kendrick 1974, Descals and Webster 1977). Stock culture slants were prepared by inoculating glass screw cap test tubes containing 0.1 % malt agar with squares of agar cut from the plates, incubating at room temperature for 3 days to initiate growth of the organisms and storing at 4°C to slow growth. Oomycete cultures were also maintained by placing an agar/hyphae square in a petri dish of distilled water with a sterile hempseed half on top of the square, grown for 1-2 weeks to create a healthy colony then stored at 4°C to decrease the metabolic rate and therefore the growth of the organisms (Johnson 1956).

Four 3 mm mineral salts plus glucose agar plugs of each fungus were cut with a cork borer inoculated into two 250 ml flasks containing 50 mls of a mineral salts plus 10 % glucose plus 9.1 mg glutamate solution. This medium was chosen because it provided essential nutrients and sugar to promote growth and was the medium that most closely resembled a natural stream

(Suberkropp et al. 1983). Without the addition of glutamate the oomycete species could not be grown successfully in solution, although they were able to grow on solid agar media containing the same concentrations of each of the nutrients and the sugar. To test the assumption that one or more amino acids were missing that might be required for growth in solution select amino acids were added to the medium in combination and individually. Glutamate addition promoted the growth of the organisms and did not appear to have any negative repercussions on the morphology of either the hyphomycetes or the oomycetes. The concentration of the glutamate was adjusted so that the minimum amount that allowed sufficient growth was added. While combinations of amino acids might have provided a similar result only one was chosen in order to maintain the simplicity of the media.

The species were grown for 5-7 days at room temperature. This period of time was selected in order to assure that the organisms were past the initial growth stage but still within the linear growth period. The exact number of days for each species depended on the rate at which they grew and the time required to reach substantial colony growth for sampling. The contents of the flask were homogenized with a waring blender and transferred to sterile centrifuge tubes. The tubes were centrifuged, the supernatant discarded, and the mycelia resuspended in 50 ml of sterile distilled water. Twenty-five 250 ml flasks per organism containing 50 ml of a mineral salts/glutamate medium plus approximately 25 mg leaf discs were inoculated with 1 ml aliquots of the appropriate fungal suspension. Flasks were maintained stationary at room temperature for 3, 7, 14, 21, and 28 days. At each harvest 3 or 4 flasks per organism were filtered to separate the mycelia and leaf discs from the medium. The volume and pH of the media were measured immediately. Sample media were then frozen and lyophilized. Lyophilization was the preferred method since it allowed for drying of the samples by sublimation rather than the application of heat which could cause denaturing of the enzymes to be assayed. The resulting dry matter was weighed, resuspended in 10 ml of 50 mM, pH 5 acetate buffer, and again frozen until ready for enzyme assays. The mycelia plus leaf material was frozen, lyophilized and weighed. Sample leaf material was then homogenized in 5 ml of 50 mM, pH 5 acetate buffer and frozen. In preparation for enzyme assays, both the supernatant and homogenate were thawed and 0.01 % thimerosol added to inhibit bacterial growth. The material was then refrigerated at 5°C.

2. Enzyme Assay Methods

The enzymes chosen for study included endocellulase (ß-1,4,-endoglucanase : EC3.2.1.4), exocellulase (ß-1,4,-exoglucanase : EC3.2.1.91), pectin lyase, and polygalacturonase. This selection was based upon the structural makeup of leaf litter and the known ability of several hyphomycete and a few oomycete fungi to break down and utilize some of these components (Alberts et al. 1989, Barlocher and Kendrick 1975, Berner and Chapman 1977, Chamier 1985, Deacon 1975, Miele and Linkins 1974, Suberkropp and Arsuffi 1984, Thompstone and Dix 1985). To assay for the enzymes pectin lyase and polygalacturonase, 2-3 mls of the homogenate supernatant and the media were dialyzed against distilled water for 24 hours with a change of water after 12 hours. The dialyzation was necessary to remove sugars that might interfere with measurement of these enzymes. Dialyzation was not necessary for the endocellulase and exocellulase enzyme analyses. In a second growth study, performed in the Fall of 1991, the volume of media utilized was 20 ml. The decreased media volume was used in order to concentrate the enzymes produced and increase the detection limits. Several flasks were also maintained on a shaker table and harvested after 14 and 21 days during the Fall 1991 growth study. A full sampling regime was not temporally feasible and the dates were selected because this is often the time period during which organisms reach their maximium rate of growth and/or enzyme production under laboratory controlled conditions.

a. Endocellulase Assay (Bernfield 1955, Arsuffi and Suberkropp 1988)

To assay for endocellulase activity 0.2 ml of the sample was combined with 0.2 ml of 0.5% carboxymethyl cellulose suspended in 50 mM, pH 5 acetate buffer to which 0.01 % thimerosol had been added. Samples and controls (enzyme added after incubation) were incubated at 30°C for 3 hrs on a shaker table. The amount of reducing sugars produced was determined by the Nelson-Somogyi Method (Somogyi 1952, Nelson 1944) as follows. The 0.4 ml reaction mixture was combined with 0.4 ml of the combined copper reagent as decribed below. Tubes were vortexed and heated in a boiling water bath for 20 minutes then cooled in a pan of cold water. To each tube 0.4 ml of the arsenomolybdate reagent was added with vortexing (see below). Three ml of dH_2O was added and after vortexing, samples were read at 520 nm on a spectrophotometer. Activity (ug glucose liberated/ug protein/hr) was determined by comparing absorbances with standard curves prepared using known concentrations of glucose.

b. Exocellulase Assay (Sinsabaugh et al 1991, Bernfield 1955, Arsuffi and Suberkropp 1988)

The assay for exocellulase activity involved combining 0.2 ml of the sample with 0.2 ml 2% microcrystalline cellulose suspended in 50 mM, pH 5 acetate buffer (0.01 % thimerosol added). Control tubes were centrifuged immediately and 0.2 ml of the supernatant was withdrawn. Samples and controls were incubated on a shaker table for 18-20 hrs at 30°C. After the incubation period sample tubes were centrifuged and 0.2 ml of the supernatant was withdrawn and pipetted into clean test tubes. Reducing sugars were determined by the Nelson-Somogyi method as described above. Samples were read at 520 nm on a spectrophotometer. Activity (ug glucose/ug protein) was determined by comparing absorbances with standard curves prepared using known concentrations of glucose.

c. Pectin Lyase Assay (Anderson 1978)

To assay for pectin lyase enzyme activity, 0.2 ml of dialyzed sample was combined with 0.2 ml of 0.05 M bicine buffer (pH 8) plus 0.03 M CaCl2 and 0.2 ml 0.5% pectin (dialyzed and centrifuged). Samples and controls (enzyme added after incubation) were incubated on a shaker table at 30° C for 3 hours. Reducing sugars were determined by the Nelson-Somogyi method described above. Samples were read at 550 nm on the spectrophotmeter. Activity (ug galacturonic acid liberated/ug protein/hr) was determined by comparing absorbances with standard curves prepared by using known [galacturonic acid].

d. Polygalacturonase Assay (Bernfield 1955, Arsuffi and Suberkropp1988)

For the polygalacturonase enzyme assay 0.2 ml of dialyzed sample was added to 0.2 ml 0.5% polygalacturonic acid (grade II, Sigma) and 0.2 ml of 50 mM, pH 5 acetate buffer, pH 5. Samples and controls (enzyme added after incubation) were incubated on a shaker at 30°C for 3 hours. Reducing sugars were determined by the Nelson-Somogyi method. Tubes were read at 515 nm on a spectrophotometer. Activity (ug galacturonic acid liberated/ug protein/hr) was determined by comparing absorbances with standard curves prepared using known [galacturonic acid].

3. Bio-Rad Protein Assay (Bradford 1976, Kley and Hale1977)

A modified version of the protein dye-binding method described by Bradford, 1976 using coomassie blue dye was utilized to measure the amount of protein in the culture medium as well as in the resultant supernatant after homogenization of the leaf discs. Several dilutions of bovine serum albumin (BSA) protein standard containing from 1 to 25 ug/ml were prepared. 0.4 ml of standards, samples, and blanks (dH2O) were placed into clean, dry test tubes. To each test tube 0.1 ml concentrated dye reagent was added and vortexed. After 5 to 60 minutes 2 ml of dH_2O was added to each tube, vortexed and measured at 595 nm on a spectrophotometer. The concentration of protein in the samples was determined by extrapolation from the resulting BSA standard curve.

4. Reducing Sugar Determination (Somogyi 1952 and Nelson 1944)

Equal volumes of the sample/enzyme reaction mixture and a combined copper reagent (see Appendix Two for details) are added to a test tube, vortexed and heated for 20 minutes in boiling water bath. Tubes are removed and cooled in a pan of cold water. To the cooled tubes a volume of arsenomolybdate reagent (see Appendix Two for details) comparable to that of the sample is added with vortexing. Tubes are diluted with distilled water, vortexed and read on a spectrophotometer at the appropriate wavelength for the particular enzyme in question.

RESULTS

1. Growth Study

Figure 9 shows the average (± 1 S.E.) pH pattern for each species compared with a control consisting of leaf discs alone. Although the original pH of the media was 7.0, the autoclaving process reduced the pH. Five flasks containing media alone were autoclaved and the average pH calculated to be 6.63 ± 0.17. In general, the flasks inoculated with the fungal species showed increased pH with time compared to the control flasks which showed a slight decline over time. Observations of the pH pattern over the course of the experiment aids in the evaluation of the enzymes that are produced as well as the robustness of a colony. It has been recorded that certain enzymes are more active at different pH's. For example, pectin lyase is released under alkaline conditions and polygalacturonase, although a constitutive enzyme, is more active under acidic conditions (Chamier and Dixon, 1982; Chamier, 1985; Miller and MacMillan, 1970; Suberkropp et al, 1983). The pH of all species declined after 28 days of incubation possibly due to a build up of phenolics in the media stunting the growth of the organisms. A second growth study conducted with four of the original six species presented a similar pattern (Figure 10). Both studies showed the initial media pH of *Tetracladium marchalianum* to be significantly higher than the other organisms. This may be an indication that *T. marchalianum* begins growing within the first three days after inoculation. Visual observations of the organisms during the first growth study indicated a slower rate of growth for both *Dictyuchus monosporus* and *Triscelophorus monosporus*.

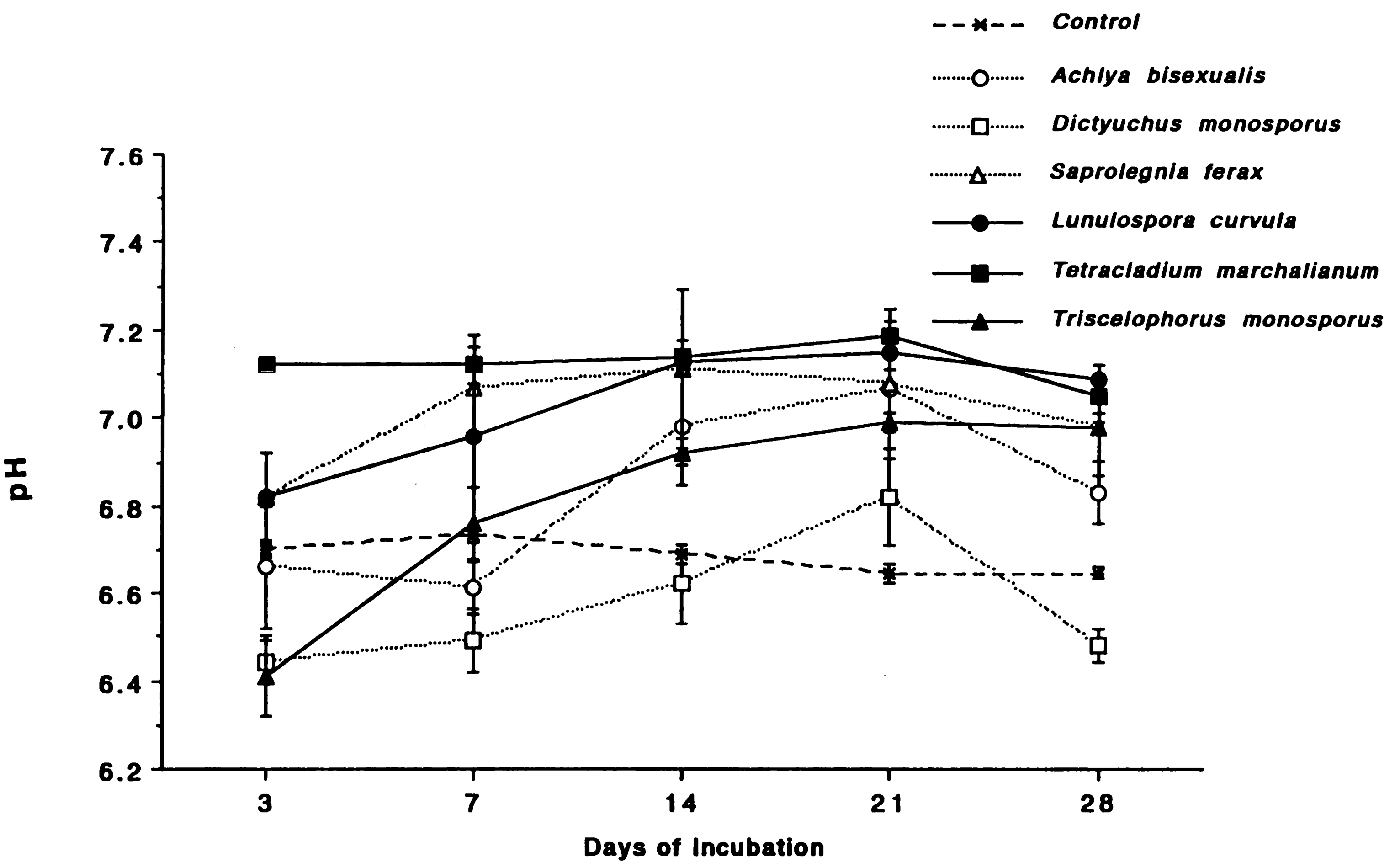


Figure 9 Spring Growth Study pH Data for the Selected Hyphomycetes (solid symbols) and Oomycetes (open symbols). Error bars = +/- 1 S.E.

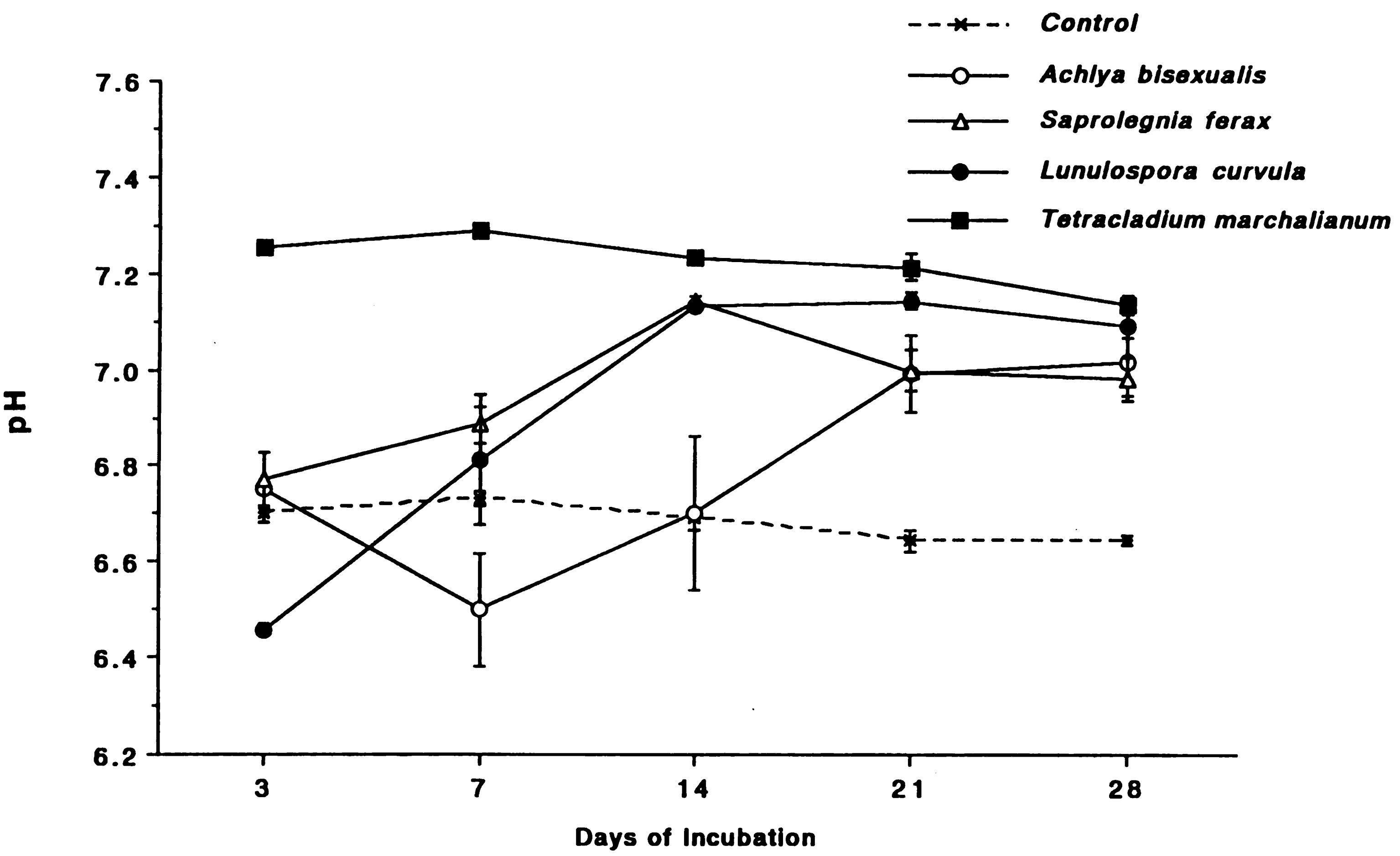


Figure 10 Fall Growth Study pH Data for Four of the Six Selected Hyphomycetes (solid symbols) and Oomycetes (open symbols). Error bars = +/- 1 S.E.

Figures 11 and 12 show the protein concentration in the undialyzed and the dialyzed medium and supernatant of the homogenate. Samples were dialyzed to remove large sugar molecules that cause interference during analysis of the pectic enzymes. Protein determinations were conducted to ensure a minimal loss of enzyme concentration due to dialysis. Since the concentration of protein was used in the calculations of the amount of the various enzymes observed, awareness of this parameter was important. Dialysis did not appear to have much of an affect on the concentration in either the medium or the homogenate supernatant. Both figures indicate that the greatest concentration of protein was detected in the *L. curvula* cultures especially after 7 and 14 days. This may be an indication of greater enzyme activity by this organism, as will be shown in subsequent sections. Increased protein would be expected as microbes immobilize nitrogen (Suberkropp et al 1976) but can be confounded by protein complexing or other compounds, such as phenols.

Paired t-tests of mean protein concentration within the medium versus the supernatant of the homogenate showed there were no significant differences for any of the organisms except *D. monosporus* (Table 13). The concentration of protein in the homogenate of *D. monosprous* was significantly lower than in the medium, possibly an indication that this organism may be producing enzymes that cannot penetrate the leaf and are thus being released into the medium. Medium protein was correlated with supernatant homogenate protein for only three of the six organisms. Changes in the concentration of protein in the medium paralled changes in the homogenate for the organisms *A. bisexualis, S. ferax,* and *L. curvula.* An ANOVA of protein concentration during the incubation period showed significant differences between species at all dates in the medium, except after 28 days, and for all dates in the supernatant

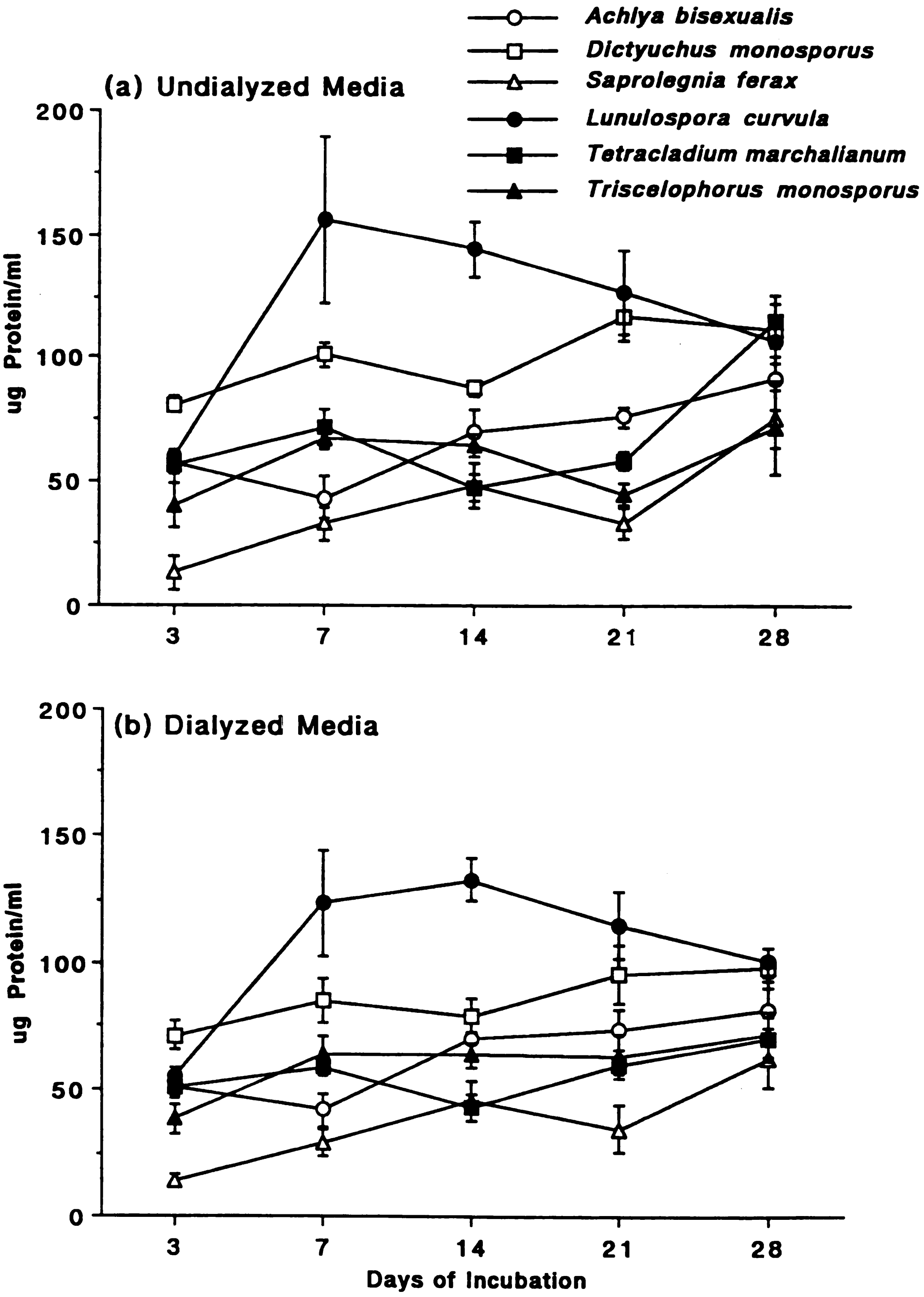


Figure 11 Protein Concentration (ug/ml) of the Undialyzed (a) and the Dialyzed (b) Spring 1990 Media. Error bars = +/- 1 S.E.

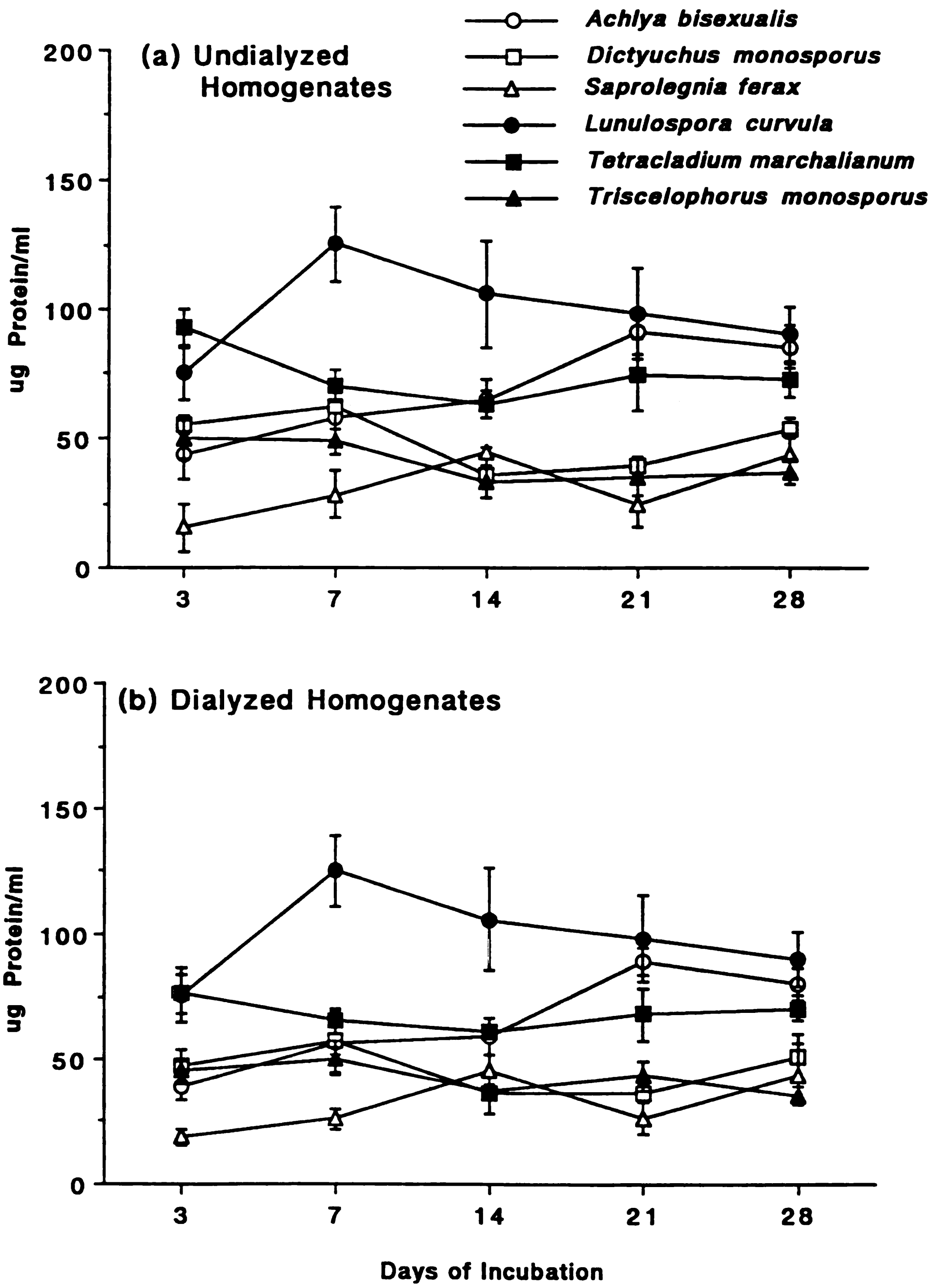


Figure 12 Protein Concentration (ug/ml) of the Undialyzed (a) and Dialyzed (b) Spring 1990 Supernatant Homogenate. Error bars = +/- 1 S.E.

Table 13 Paired t-tests and correlations of mean protein concentration within the media versus the homogenates during the 28 day spring 1991 growth study, N=5.

Species	Paired t-value	probability	Correlation Coefficient
Achlya bisexualis	-0.205	0.848	0.763*
Dictyuchus monosporus	5.777	0.005	NS
Saprolegnia ferax	1.570	0.191	0.896**
Lunulospora curvula	2.088	0.105	0.944**
Tetracladium marchalianum	-0.367	0.732	NS
Triscelophorus monosporus	2.116	0.102	NS

* significance $p < 0.05$
** significance $p < 0.01$

Table 14 ANOVA of mean protein concentration in media and the homogenates between species with days as the independent variable, N=6.

	Media		Homogenates	
Incubation days	F-value	Probability	F-value	Probability
3	15.66	0.0001	10.17	0.0005
7	8.84	0.0010	14.29	0.0001
14	22.40	0.0001	7.46	0.0021
21	18.46	0.0001	8.43	0.0031
28	2.20	0.0999	8.44	0.0003

homogenate (Table 14). Scheffe's f-test (Table 15) indicates that the differences are mainly due to significantly lower concentrations for *Saprolegnia ferax* after 3 days, higher concentrations for *Lunulospora curvula* after 7, 14, and 21 days, and lower concentrations of *S. ferax* and *Triscelophorus monosporus* (only in the supernatant homogenate) after 18 days.

2. Enzyme Production

The structure of plant cell wall as described by Chamier (1985) indicates a need for organisms to produce enzymes that will degrade cellulose, pectin, and hemicellulose. Cellulose is found as microfibrils noncovalently bonded to hemicellulose and interconnected by pectic polysaccharides.

a. Endocellulase Activity (Endo)

Activity of the endocellulase enzyme, also referred to as endoglucanase, measures the ability of a fungus to degrade soluble (amorphous) cellulose. The action of this enzyme is to break the ß-1,4-glycosidic bonds that link glucose units to one another with the ability to penetrate deep into the cellulose matrix and therefore break bonds that are inaccessible by other enzymes. However, the complete degradation of native cellulose requires a synergism between endocellulase and two other cellulose degrading enzymes, exocellulase and cellobiose. Figure 13 illustrates the superior ability of *L.curvula* to produce this enzyme compared to the other two hyphomycetes, *T. marchalianum* and *T. monosporus*, as well as the oomycetes, *A. bisexualis, D. monosporus,* and *S. ferax*. These oomycetes were able to produce a level of enzyme comparable to the two hyphomycetes, especially in the medium. Enzyme activity in the homogenate showed relatively the same pattern but not to the same magnitude.

Table 15 Significant species comparisons of mean protein concentration and their f values as determined by Scheffe's f-test.

	Media		Homogenates	
Incubation days	Comparison	f value	Comparison	f value
3	Ab vs Sf	5.84	Ab vs Tma	3.42
	Dm vs Sf	13.92	Sf vs Lc	5.16
	Dm vs Tmo	4.97	Sf vs Tma	8.53
	Sf vs Lc	6.66		
	Sf vs Tma	5.62		
7	Ab vs Lc	5.59	Ab vs Lc	6.17
	Sf vs Lc	6.71	Dm vs Lc	5.34
	Tma vs Lc	3.14	Sf vs Lc	12. 61
	Tmo vs Lc	3.47	Tma vs Lc	4.11
			Tmo vs Lc	7.82
14	Ab vs Lc	9.42	Sf vs Lc	3.83
	Sf vs Lc	15.79	Dm vs Lc	4.98
	Dm vs Lc	5.57	Tmo vs Lc	5.42
	Tma vs Lc	16.08		
	Tmo vs Lc	10.89		
21	Ab vs Lc	3.21	Ab vs Sf	3.83
	Dm vs Sf	8.76	Sf vs Lc	4.65
	Dm vs Tma	4.33	Tmo vs Lc	3.36
	Dm vs Tmo	6.51		
	Sf vs Lc	10.95		
	Tma vs Lc	5.90		
	Tmo vs Lc	8.41		
28			Ab vs Sf	2.96
			Ab vs Tmo	3.99
			Sf vs Lc	3.69
			Lc vs Tmo	4.23

Ab = *Achlya bisexualis*
Dm = *Dictyuchus monosporus*
Sf = *Saprolegnia ferax*
Lc = *Lunulospora curvula*
Tma = *Tetracladium marchalianum*
Tmo = *Triscelophorus monosporus*

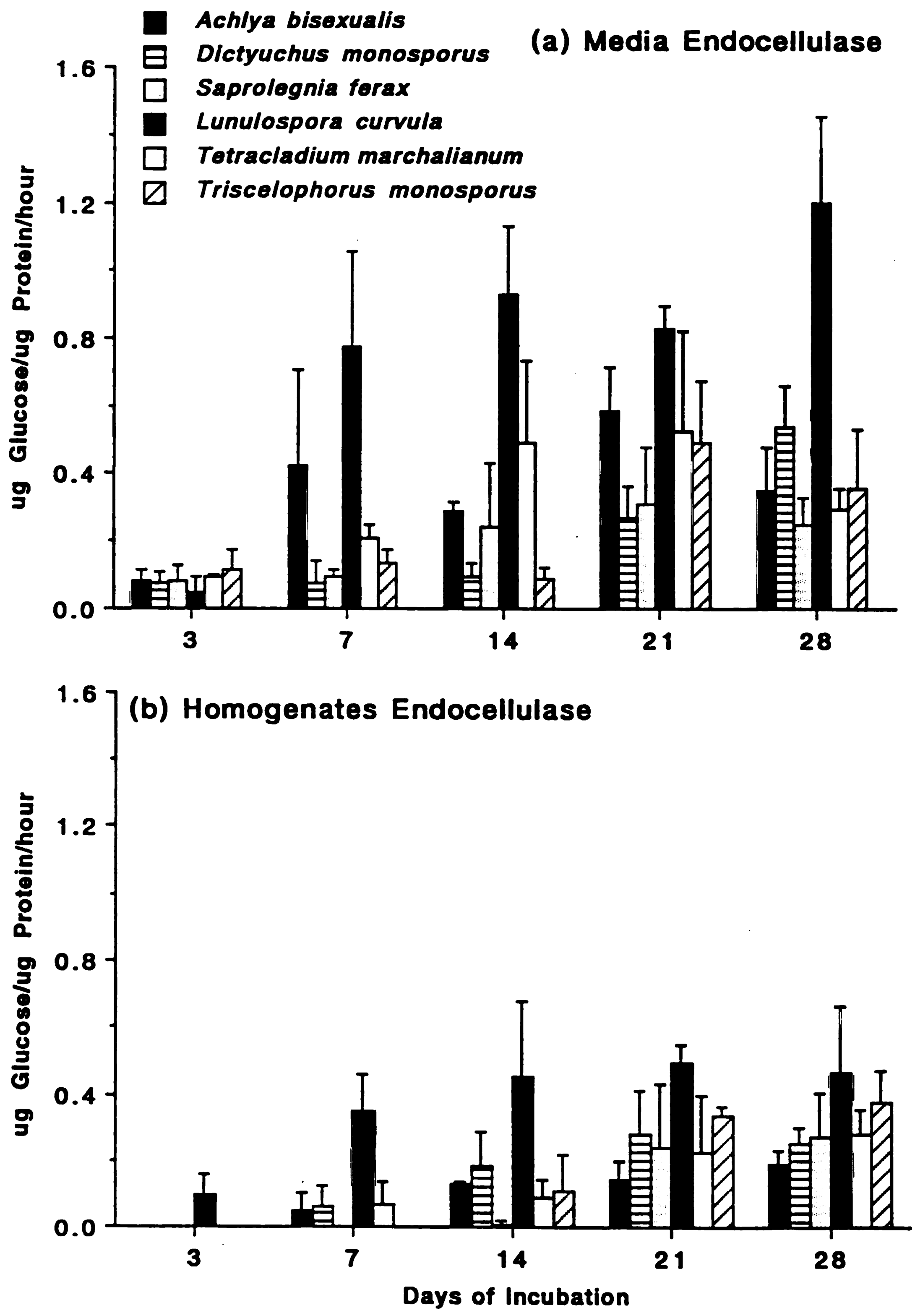


Figure 13 Endocellulase Activity in the Media (a) and Homogenates (b) from the Spring 1990 Growth Study. Error bars = +/- 1 S.E.

Endocellulase activity within the medium and the supernatant homogenate of the selected species was compared using a one-factor ANOVA (Table 16). Results indicated significant differences between species when mean and total activity at each sampling date was compared. When each sampling date was analyzed separately using the one-factor ANOVA a significant difference was detected at 14 and 28 days for the medium and at 3 and 7 days for the homogenate. Scheffe's F-test revealed that the differences in both the media and the homogenates were due mainly to greater activity by *Lunulospora curvula* (Table 17). Although the data do not show significant results at other times, *L. curvula* appears to always have the highest activity in both the medium and the homogenate (Figure 13a, b). In general, enzyme activity for all the organisms was greater in the media than in the homogenates. Kruskal-Wallis rank data of the media showed *Triscelophorus monosporus* to have the greatest activity after 3 days and *L. curvula* after 7, 14, 21 and 28 days (Table 18). Overall mean activity was ranked first for *L. curvula* followed by *A. bisexualis, T. marchalianum, D. monosporus, T. monosporus* and finally *S. ferax*. For the homogenate activity, *L. curvula* had the highest activity at all dates (Table 19). Overall mean ranks placed *L. curvula* first followed by *T. marchalianum, D. monosporus, T. monosporus, A. bisexualis* then *S. ferax.* Ranking for endocellulase activity suggests that two of the three hyphomycetes ranked higher than any of the three oomycetes.

b. Exocellulase Activity (Exo)

The exocellulase enzyme (exoglucanase) degrades crystalline cellulose and it's mode of action is to clip off glucose units from the ends of cellulose chains. The hyphomycete *T. marchalianum* had the highest exocellulase activity in the media, especially after 14 and 21 days (Figure 14a). The

Table 16 One - Factor Analysis of Variance between selected species for study and production of selected enzymes.

Incubation Days	df	Endo	Exo	PGA	PL
Media					
3	17	0.241	2.390	2.227	-----
7	17	2.665	2.499	3.340*	5.704*
14	17	4.296*	8.058*	0.800	6.519*
21	17	1.505	20.181*	4.509*	1.201
28	23	5.613*	2.007	0.639	0.659
Means	29	3.972*	3.417*	0.469	1.615
Overall	95	8.016*	7.207*	1.029	2.605*
Homogenates					
3	17	3.119*	-----	3.205*	3.996*
7	17	4.614*	1.413	2.740	3.411*
14	17	1.872	1.508	12.615*	1.937
21	17	0.978	1.965	5.030*	1.875
28	23	0.789	1.770	1.889	1.378
Means	29	2.713*	1.321	1.457	0.709
Overall	95	4.189*	2.544*	3.472*	1.532

* significant at the $p \leq 0.05$ level

Significant Scheffe F-test Results

Medium : Pectin Lyase - *Lunulospora curvula* was significantly higher than all the other organisms after 7 and 14 days

Exocellulase - *Tetracladium marchalianum* was significantly higher than the other organisms on days 14 and 21 except *L.curvula* on day 14.

Polygalacturonase - *Achlya bisexualis* showed significantly greater activity than *Saprolegnia ferax* at day 21.

Endocellulase - *Lunulospora curvula* had significantly higher activity after 28 days compared to the other species except *Dictyuchus monosporus*. A comparison of the means at each sampling date showed *L. curvula* to be greater than *S. ferax* and *D. monosporus*.

Homogenate : Polygalacturonase - After 14 days *L. curvula* was signicantly greater than all the other organisms.

Endocellulase - *L.curvula* showed higher activity than *S. ferax* and *Triscelophorus monosporus* after 7 days.

Table 17 Significant species comparisons of endocellulase activity based upon Scheffe's F-test with values being significant at the 95% level.

Comparison	Overall	Mean	3	7	14	21	28
Media							
Lc vs Sf	5.576	2.812	--	--	--	--	3.963
Lc vs Ab	3.131	--	--	--	--	--	3.163
Lc vs Dm	4.992	2.675	--	--	--	--	--
Lc vs Tma	3.495	--	--	--	--	--	3.607
Lc vs Tmo	5.374	--	--	--	--	--	3.116
Homogenates							
Lc vs Sf	2.871	--	--	3.261	--	--	--
Lc vs Ab	3.043	--	--	--	--	--	--
Lc vs Tmo	--	--	--	3.261	--	--	--

Ab = *Achlya bisexualis*
Dm = *Dictyuchus monosporus*
Sf = *Saprolegnia ferax*
Lc = *Lunulospora curvula*
Tma = *Tetracladium marchalianum*
Tmo = *Triscelophorus monosporus*

Table 18 Results of the Kruskal-Wallis rank test on the enzyme activity within the media of the selected species.

Days	Species	Endo	Exo	PGA	PL
3	H value	0.86	10.03	10.59	0
	A. bisexualis	10.00	14.83*	8.50	9.50
	D. monosporus	9.00	12.67	8.50	9.50
	S. ferax	9.33	11.33	8.50	9.50
	L. curvula	7.33	7.83	14.50*	9.50
	T. marchalianum	10.33	7.67	8.50	9.50
	T. monosporus	11.00*	2.67	8.50	9.50
7	H value	9.61	9.11	8.03	16.83
	A. bisexualis	11.33	11.17	10.33	8.00
	D. monosporus	4.67	10.00	8.00	8.00
	S. ferax	6.00	5.67	9.33	8.00
	L. curvula	16.30*	9.00	16.00*	17.00*
	T. marchalianum	11.00	16.30*	8.33	8.00
	T. monosporus	7.67	4.83	5.00	8.00
14	H value	11.15	11.18	4.97	16.83
	A. bisexualis	11.67	5.50	9.83	8.00
	D. monosporus	5.00	4.50	10.83	8.00
	S. ferax	7.33	7.67	10.00	8.00
	L. curvula	16.33*	12.67	11.33	17.00*
	T. marchalianum	12.00	16.67*	11.50*	8.00
	T. monosporus	4.67	10.00	3.50	8.00
21	H value	6.31	10.08	11.66	5.23
	A. bisexualis	11.67	9.67	16.33*	8.17
	D. monosporus	6.33	4.00	9.67	7.33
	S. ferax	6.67	8.00	2.33	8.00
	L. curvula	15.00*	11.00	7.00	14.33*
	T. marchalianum	10.33	17.00*	12.00	12.17
	T. monosporus	7.00	7.33	9.67	7.00
28	H value	10.31	9.47	4.60	2.50
	A. bisexualis	10.63	7.75	17.00*	9.88
	D. monosporus	15.50	7.50	17.00*	9.88
	S. ferax	8.25	11.25	12.50	14.63
	L.curvula	21.50*	20.38*	11.75	15.63*
	T. marchalianum	9.63	13.00	7.25	13.88
	T. monosporus	9.50	15.13	11.25	10.38
Total	H value	18.62	18.78	4.23	14.36
	A. bisexualis	52.50	46.41	55.47*	44.56
	D. monosporus	39.88	34.81	49.72	43.53
	S. ferax	37.00	39.28	41.41	44.03
	L.curvula	72.25*	58.25	54.28	69.28*
	T. marchalianum	51.28	70.66*	49.50	47.56
	T. monosporus	38.09	41.59	40.63	42.03
Mean	H value	6.45	8.08	2.35	5.49
	A. bisexualis	17.20	14.80	18.20*	13.40
	D. monosporus	10.60	9.00	16.00	13.00
	S. ferax	11.80	12.80	12.80	14.30
	L.curvula	23.00*	18.40	17.40	22.70*
	T. marchalianum	16.40	23.60*	17.00	16.70
	T. monosporus	14.00	14.40	11.60	12.90

* highest value within a particular comparison

Table 19 Results of the Kruskal-Wallis rank test on the enzyme activity within the homogenates for the selected species.

Days	Species	Endo	Exo	PGA	PL
3	H value	16.83	0	10.59	10.59
	A. bisexualis	8.00	0	8.50	8.50
	D. monosporus	8.00	0	8.50	8.50
	S. ferax	8.00	0	8.50	8.50
	L. curvula	17.00*	0	14.50*	14.50*
	T. marchalianum	8.00	0	8.50	8.50
	T. monosporus	8.00	0	8.50	8.50
7	H value	9.77	10.41	13.70	10.59
	A. bisexualis	8.67	7.00	12.67	8.50
	D. monosporus	9.33	9.67	7.00	8.50
	S. ferax	6.50	7.00	7.00	8.50
	L. curvula	16.33*	16.00*	16.33*	14.50*
	T. marchalianum	9.67	10.33	7.00	8.50
	T. monosporus	6.50	7.00	7.00	8.50
14	H value	6.76	8.71	13.07	16.83
	A. bisexualis	10.67	4.00	6.50	8.00
	D. monosporus	10.83	13.67*	6.50	8.00
	S. ferax	4.67	5.67	9.00	8.00
	L. curvula	15.00*	11.33	17.00*	17.00*
	T. marchalianum	8.17	9.33	11.50	8.00
	T. monosporus	7.67	13.00	6.50	8.00
21	H value	4.61	8.05	12.43	9.73
	A. bisexualis	6.67	5.67	6.17	5.33
	D. monosporus	9.33	12.00	8.50	9.83
	S. ferax	7.83	4.00	3.50	4.00
	L. curvula	14.67*	9.67	15.67*	12.33
	T. marchalianum	7.50	14.00*	8.50	15.00*
	T. monosporus	11.00	11.67	14.67	10.50
28	H value	2.79	5.50	6.79	6.56
	A. bisexualis	8.00	9.25	8.50	8.75
	D. monosporus	13.00	15.25	11.50	9.13
	S. ferax	11.00	9.25	7.75	12.25
	L.curvula	14.50	10.50	17.00	19.63*
	T. marchalianum	13.25	12.25	12.75	14.25
	T. monosporus	15.25*	18.50*	17.50*	11.00
Total	H value	15.51	10.25	16.95	17.52
	A. bisexualis	42.88	35.56	42.94	37.94
	D. monosporus	48.22	55.13	42.50	44.03
	S. ferax	36.81	36.81	36.94	40.13
	L.curvula	71.44*	54.56	71.31*	69.63*
	T. marchalianum	43.94	53.58	47.13	52.06
	T. monosporus	47.72	55.56*	50.19	47.22
Mean	H value	7.98	4.86	6.42	4.38
	A. bisexualis	12.40	10.30	13.60	13.60
	D. monosporus	15.10	18.30	13.20	14.10
	S. ferax	11.60	11.20	11.40	12.20
	L.curvula	25.20*	16.10	23.60*	21.80*
	T. marchalianum	13.90	18.90*	15.40	16.50
	T. monosporus	14.80	18.20	15.80	14.80

* Species with the highest enzyme activity within a particular comparison

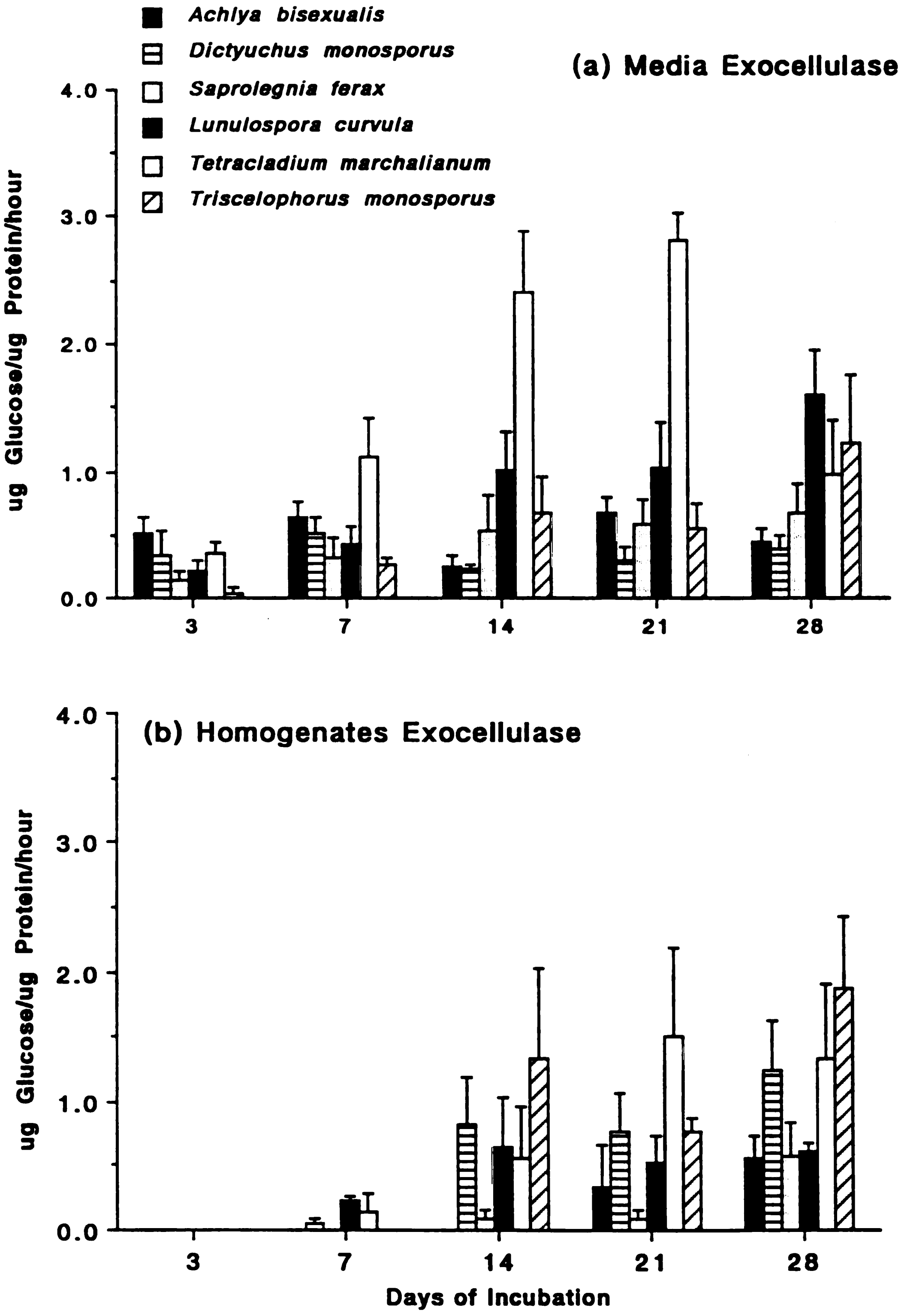


Figure 14 Exocellulase Activity in the Media (a) and Homogenates (b) from the Spring 1990 Growth Study. Error bars = +/- 1 S.E.

hyphomycetes *L.curvula* and *T. monosporus* seem to lag in the production of exocellulase and appear to begin peaking around day 28. In general, the oomycetes excreted less exocellulase into the media than the hyphomycetes. Activity detected in the homogenates (Figure 14b) was usually lower than in the media except for the hyphomycete *T. monosporus* and the oomycete *D. monosporus.* Both organisms had substantially more exocellulase in the homogenate after 14 days.

One-factor ANOVA of exocellulase activity indicated significant differences in the media when mean and total activity was compared and after 14 and 21 days as a result of much higher activity by *Tetracladium marchalianum* (Table 16). Results from Scheffe's F-test support this finding (Table 20). *Lunulospora curvula* and *Triscelophorus monosporus*, however, appear to dominate after 28 days due to a decline in enzyme concentration by *T. marchalianum* (Figure 14a). None of the oomycetes showed consistently greater exocellulase activity in the media than the other species during any of the time periods. A one-factor ANOVA of the homogenates showed there were no significant differences between species after any of the incubation periods, although total activity was significant. Scheffe's F-test was not significant for any of the comparisons. However, as is evident by Figure 14b, *Triscelophorus monosporus* shows slightly greater activity in the homogenate than the other organisms after 14 and 28 days and *Tetracladium marchalianum* is greatest after 21 days. Of the oomycetes *Dictyuchus monosporus* appears to have the greatest activity especially at 28 days. According to Kruskal-Wallis rank data (Table 18) of the medium, *Achlya bisexualis* ranked first after 3 days, *T. marchalianum* after 7, 14 and 21 days, and L. curvula after 28 days. Overall mean ranks placed *T. marchalianum* first followed by *L. curvula, T. monosporus, A. bisexualis, S. ferax and D. monosporus.* Ranks of the homogenate showed

Table 20 Significant species comparisons of exocellulase activity based upon Scheffe's F-test with values being significant at the 95% level.

Comparison	Overall	Mean	3	7	14	21	28
<u>Media</u>							
Tma vs Sf	4.281	--	--	--	4.289	11.749	--
Tma vs Ab	3.995	--	--	--	5.644	10.819	--
Tma vs Dm	5.222	--	--	--	5.731	15.009	--
Tma vs Lc	--	--	--	--	--	7.565	--
Tma vs Tmo	3.281	--	--	--	3.604	12.104	--

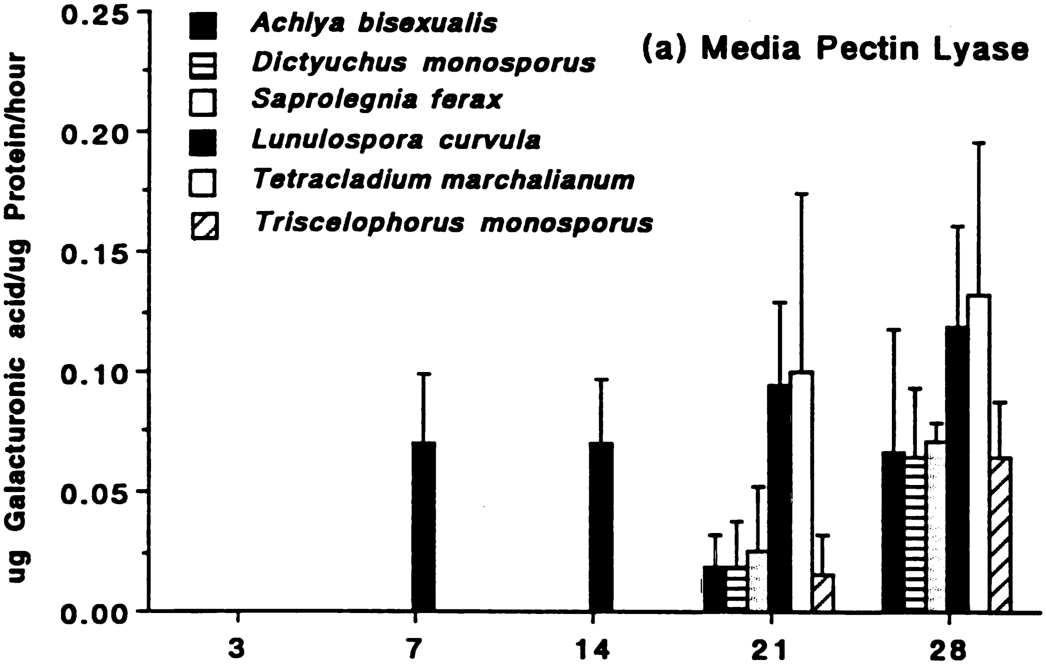
Ab = *Achlya bisexualis*
Dm = *Dictyuchus monosporus*
Sf = *Saprolegnia ferax*
Lc = *Lunulospora curvula*
Tma = *Tetracladium marchalianum*
Tmo = *Triscelophorus monosporus*

L. curvula highest after 7 days, *T. marchalianum* after 21 days, and *T. monosporus* after 14 and 28 days (Table 19). Overall, *T. monosporus* ranked first followed by *T. marchalianum, D. monosporus, L. curvula, A. bisexualis*, then *S. ferax.* Thus, all three hyphomycetes ranked above all three oomycetes in producing exocellulase in the medium and above two of the oomycetes in producing exocellulase in the homogenate.

c. Pectin Lyase Activity (PL)

The enzyme pectin lyase, induced in the presence of pectin and pH dependent, degrades pectin (esterified galacturonic acid) by ß-elimination. Figure 15 shows pectin lyase activity to be more characteristic of the hyphomycetes. The oomycetes did not begin to show significant quantities of this enzyme until day 28 in both the medium and the homogenate. In general, the hyphomycetes *L. curvula* and *T. marchalianum* produced more pectin lyase than the hyphomycete *T. monosporus* and the three oomycetes *A. bisexualis, D. monosporus*, and *S. ferax.*

A one-factor ANOVA of the pectin lyase activity within the media and the supernatant homogenates of the selected species indicated no significant differences in mean and total activity between species (Table 16). An ANOVA of the individual values at each sampling period showed significant differences at 7 and 14 days for the medium and at 3 and 7 days for the homogenate. Scheffe's F-test (Table 21) of the media agrees with this conclusion but as Figure 15 shows, this is due to the fact that *L. curvula* was the only organism that had any activity at all. Results of Scheffe's F-test indicated that the significant differences were largely due to greater activity by *Lunulospora curvula.* As is shown in Figure 15, pectin lyase activity in the media of *L.curvula* and *T. marchalianum* appears to be greater than the other organisms after 21



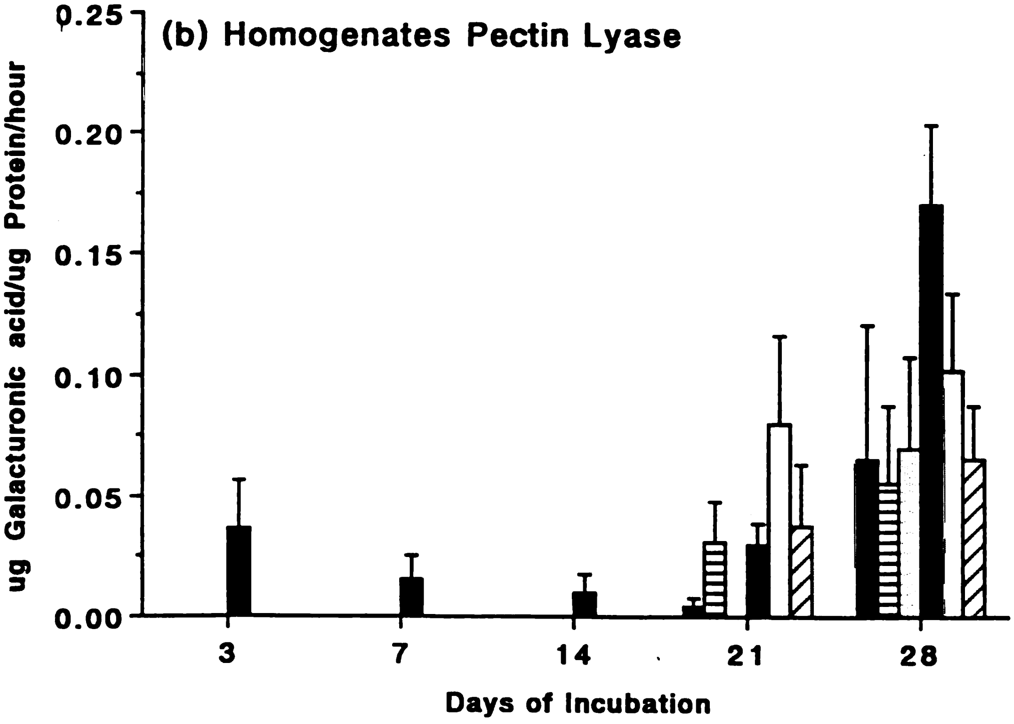


Figure 15 Pectin Lyase Activity in the Media (a) and the Homogenates (b) from the Spring 1990 Growth Study. Error bars = +/- 1 S.E.

Table 21 Significant species comparisons of pectin lyase activity based upon Scheffe's F-test with values being significant at the 95% level.

Comparison	Overall	Mean	3	7	14	21	28
<u>Media</u>							
Lc vs Sf	--	--	--	3.423	3.912	--	--
Lc vs Ab	--	--	--	3.423	3.912	--	--
Lc vs Dm	--	--	--	3.423	3.912	--	--
Lc vs Tma	--	--	--	3.423	3.912	--	--
Lc vs Tmo	--	--	--	3.423	3.912	--	--

Ab = *Achlya bisexualis*
Dm = *Dictyuchus monosporus*
Sf = *Saprolegnia ferax*
Lc = *Lunulospora curvula*
Tma = *Tetracladium marchalianum*
Tmo = *Triscelophorus monosporus*

and 28 days of incubation. In the homogenates, *T. marchalianum* dominates after 21 days while *L. curvula* exceeds all the others after 28 days. Kruskal-Wallis rank data of the media placed *L. curvula* first after 7, and 14 days and *T. marchalianum* after 21 and 28 days (Table 18). *L. curvula* was first when overall means were compared followed by *T. marchalianum, S. ferax, A. bisexualis, D. monosporus* then *T. monosporus.* However, there is very little difference between mean ranks for the four lowest organisms. Homogenate data showed the greatest activity for *L. curvula* on all dates except after 21 days when *T. marchalianum* was greater (Table 19). Overall rank data placed *L. curvula* first followed by *T. marchalianum, T. monosporus, D. monosporus, S. ferax* then *A. bisexualis.* Thus, all the hyphomycetes had greater pectin lyase activity than did the three oomycetes.

d. Polygalacturonase Activity (PGA)

The role of the constitutively produced enzyme polygalacturonase in the degradation of plant material is the hydrolysis of pectin units. Polygalacturonase activity is shown in Figure 16. The greatest activity in both the media and the homogenate occurred after 21 or 28 days for all the organisms. By day 28 the oomycete species equaled or exceeded the hyphomycete species for polygalacturonase activity in the media. Interestingly, the oomycete *A. bisexualis* exhibited the highest activity in the media after 21 and 28 days compared to the other oomycetes and the hyphomycetes. Generally, higher levels of the enzyme were found in the homogenates of the hyphomycete cultures than the oomycete cultures.

An ANOVA of polygalacturonase activity demonstrated significant results after 7 and 21 days in the media and after 3, 14, and 21 days in the homogenates (Table 16). Mean activity was not significantly different for any of

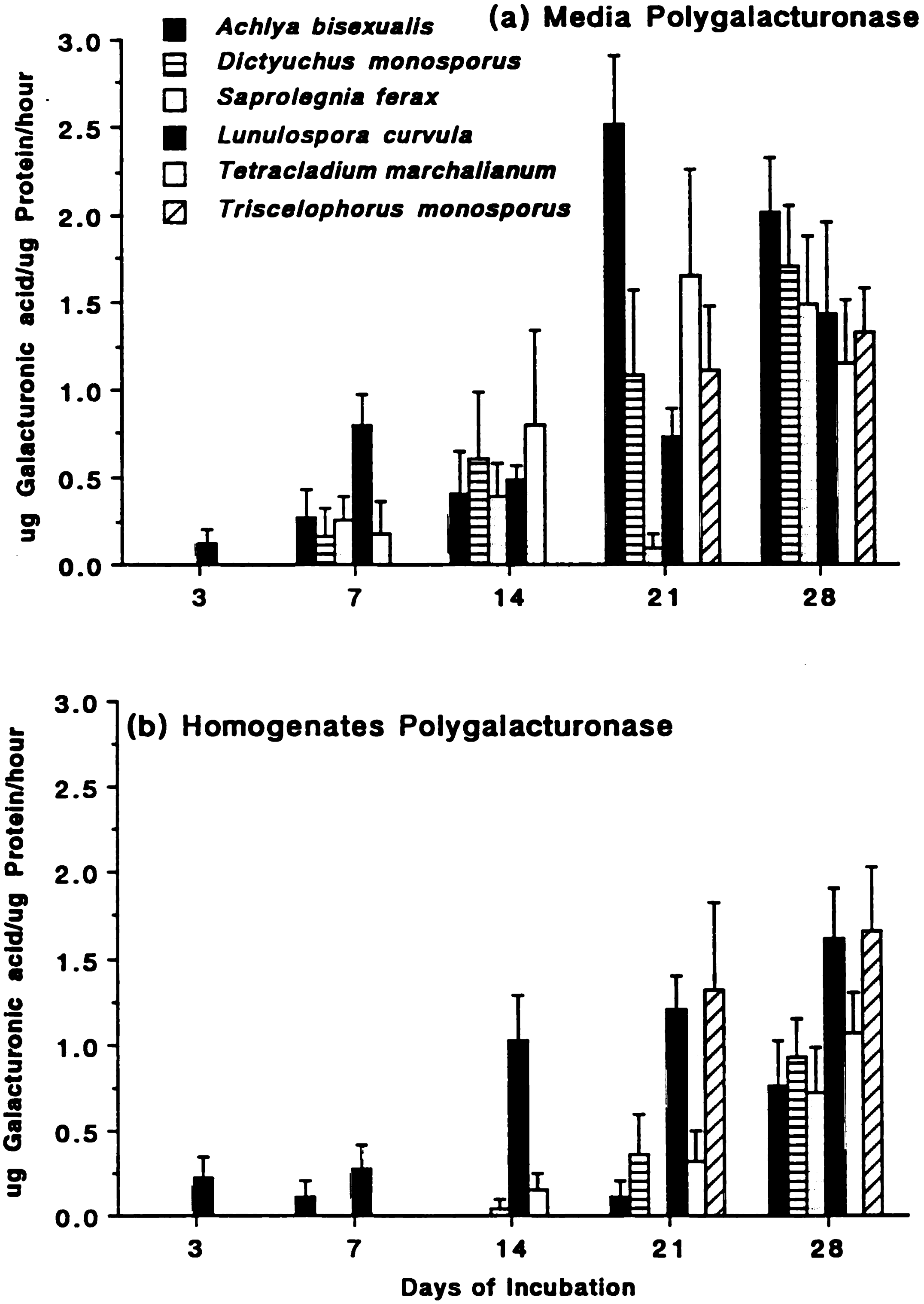


Figure 16 Polygalacturonase Activity in the Media (a) and the Homogenates (b) from the Spring 1990 Growth Study. Error bars = +/- 1 S.E.

the organisms. Higher activity by *Achlya bisexualis* in the medium at 21 days and by *Lunulospora curvula* in the homogenate at 14 days according to Scheffe's F-test were mostly responsible for the significant homogenate differences (Table 22, Figure 16). In general, the hyphomycetes *L.curvula* and *T. monosporus* had consistently higher activity in the homogenate than any of the other organisms. According to the Kruskal-Wallis rank data test of the media *L. curvula* is ranked first after 3 and 7 days, *T. marchalianum* after 14 days and *Achlya bisexualis* after 21 days and 28 days (Table 18). Overall means showed *A. bisexualis* first followed by *L. curvula, D. monosporus, T. marchalianum, Saprolegnia ferax,* then *Triscelophorus monosporus. L. curvula* showed the highest homogenate activity after 3, 7 and 14 days but after 21 and 28 days *T. monosporus* was slightly higher (Table 19). Overall *L. curvula* ranked first followed by *T. monosporus, T. marchalianum, D. monosporus, A. bisexualis,* then *S. ferax.* Overall, the three hyphomycetes again ranked ahead of the three oomycetes in polygalacturonase activity.

Table 22 Significant species comparisons of polygalacturonase activity based upon Scheffe's F-test with values being significant at the 95% level.

Comparison	Overall	Mean	3	7	14	21	28
<u>Media</u>							
Sf vs Ab	--	--	--	--	--	3.896	--
<u>Homogenates</u>							
Lc vs Sf	--	--	--	--	7.298	--	--
Lc vs Ab	--	--	--	--	8.018	--	--
Lc vs Dm	--	--	--	--	8.018	--	--
Lc vs Tma	--	--	--	--	5.851	--	--
Lc vs Tmo	--	--	--	--	8.018	–	--

Ab = *Achlya bisexualis*
Dm = *Dictyuchus monosporus*
Sf = *Saprolegnia ferax*
Lc = *Lunulospora curvula*
Tma = *Tetracladium marchalianum*
Tmo = *Triscelophorus monosporus*

DISCUSSION

Exoenzyme assays provide a functional profile of microbial activity. In this study both soluble (media) and bound (homogenates) activity were measured. Activity detected in the media gave an indication of a species ability to produce a particular enzyme, as well as the extent of enzyme production lost to the surroundings. Activity associated with the homogenates provided a clearer indication of the success of a particular species in the decomposition process. Enzymes tied to the homogenates are actively participating in the degradation of the leaf material. Leaf supernatants and extracts were assayed for extractable protein with the assumption that this protein was microbially derived (Suberkropp *et al* 1981) and reflects more closely the metabolic activity of microbes on the leaves than does total protein or nitrogen content (Sinsabaugh *et al* 1981). *L. curvula* had the highest protein concentration and peaked sooner than the other species which agrees with the observation that this species showed the greatest total enzyme activity and had greater or equivalent specific enzyme activities compared to the other species, with the exception of exocellulase activity. For other species, however, the trend was more ambiguous and is probably an indication that other enzymes were present but not assayed. For example, *Achlya bisexualis*, had moderate to high protein levels relative to the other species but when total enzyme activity was compared this species was low to moderate comparatively. Other enzymes not measured in this study, such as chitinase, xylanase, protease, are likely to be major characters in the matrix of enzymes produced by this organism. These enzymes are capable of degrading other components of the leaf, besides cellulose and pectin, as well as stream insects that feed on conditioned leaf material.

To determine if the selected species were capable of degrading cellulose, the activity of two cellulolytic enzymes was investigated. Exocellulase activity suggests an ability to breakdown highly ordered, crystalline cellulose molecules while endocellulase activity suggests an ability to degrade amorphous cellulose, both of which are components of plant cell walls. Results indicate that all species were capable of producing enzymes necessary for the degradation of the two forms of cellulose substrate. *Lunulospora curvula* showed the greatest endocellulase activity while *Tetracladium marchalianum* had the greatest exocellulase activity. These enzymes act synergistically to breakdown cellulose. However, exocellulase acts on the surface of the microfibrils and the ability of *T. marchalianum* to produce more exocellulase relative to the other species may be an indication that this organism is able to penetrate the matrix more easily and be more efficient at exploiting the amorphous cellulose. Suberkropp and Klug (1980) showed that of the hyphomycetes they examined *T. marchalianum* was the first to begin degradation of leaf material in culture and caused the greatest weight loss.

As was mentioned above, exocellulase production by *L. curvula* was lower, in most instances, than the other hyphomycetes and comparable to the oomycete species. The production of reducing sugars reflects the interaction between endo- and exocellulase activities. The endocellulase enzyme provides sites for exocellulase but generates few soluble reducing equivalents itself. When the amorphous substrate carboxymethylcellulose was utilized more sites were initially available to attack by exocellulase. So the greater "endo" activity reflected by *L. curvula* may actually be indicative of greater exocellulase plus endocellulase activity due to more non-reducing ends being available to attack. The fact that "exo" activity is low may be due more to less endocellulase activity which creates sites for exo activity. This would then agree with Sinsabaugh et al (1981) who found that leaf species with the highest protein had the lowest endocellulase activity but highest exocellulase activity. The endocellulase pattern was attributed to slower turnover rate due to tighter binding affinity to detritus. Similar results were found in the present study when different fungal species were compared on the same leaf substrate type.

If "exo" activity in this study is considered as the interaction of the two enzymes, it may be appropriate to conclude that *T. marchalianum* showed greater endo:exo activity than the other species as is evident by the greater amount of exocellulase activity in the media at 7, 14, 21 days. Golladay and Sinsabaugh (1991) found that exocellulase on leaves peaked 1-2 weeks earlier than endocellulase activity. Using the above definition of endocellulase and exocellulase activity in the present study similar results were found indicating endocellulase activity lagging approximately 1-2 weeks behind exocellulase activity.

Berner and Chapman (1977) examined the cellulolytic activity of 6 oomycetes and found that most were able to hydrolyze CMC. Also, only half of the mycelia was produced in the media with no CMC as compared to media with CMC. Thompstone and Dix (1985) examined 27 isolates of *Achlya* and *Saprolegnia* and found that almost all showed cellulase activity by their ability to reduce the viscosity of CMC and release reducing sugars from CMC. Zare-Maivan and Shearer (1988) found that oomycete species isolated from wood could produce extracellular exoglucanase, endoglucanase, and glucosidase when grown in a crystalline cellulose medium. Similar trends are apparent for the oomycetes in this study. Also, there seems to be less discrepancy between oomycete and hyphomycete celluloytic activity in the homogenate suggesting that oomycetes are as effective as hyphomycetes at degrading cellulose.

Pure culture studies of aquatic hyphomycetes, including *T. marchalianum*, on leaves by Suberkropp and Klug (1980) showed that these organisms were able to produce enzymes that hydrolyze CMC, xylan and PGA. Pectin-degrading ezymes are implicated in the softening of the leaf matrix. The ability of fungi to produce pectin-degrading enzymes was examined in this study. Most species showed a delayed response in elaboration of PGA and pectin lyase, except *L. curvula* which was able to produce these enzymes within 3-7 days. Pectin lyase activity was lower and later than expected based on evidence from the literature that this is one of the first enzymes to be elaborated during degradation of leaf material. However, this may be a reflection of the methods employed. More polygalacturonase was apparent and was elaborated sooner than pectin lyase. Interestingly, the levels exuded into the media by the oomycetes were comparable if not higher than the hyphomycetes. However, the homogenate shows that very little of that produced by the oomycetes was retained on the leaf until day 28. While all species were able to produce polygalacturonase and pectin lyase, the soluble and bound activity of these enzymes was more consistent among the hyphomycete species. The oomycete *Achlya bisexualis* produced the highest amounts of polygalacturonase into the media but relatively little of this enzyme was bound to the leaf until late in the study period. This species may not be as effective at degrading pectin during the initial stages of decomposition as one might conclude by just analyzing the activity within the medium. The homogenate data seems to indicate that Achlya was not effectively decomposing pectin during the first 2 to 3 weeks of the incubation period. Suberkropp *et al* (1983) showed that polygalacturonase was low in *T. marchalianum.* Results of this study generally agree. Relative to the other hyphomycetes, *T. marchalianum* produced less bound enzyme activity.

Butler (1984) and Butler and Suberkropp (1986) concluded that levels of enzyme activity were not related to the ability of the fungus to grow, cause weight loss, or soften leaves. However, this may be due to analysis of the media only. Enzyme activity measured in the homogenate (bound) may be the better predictor for degradative capability. In the present study for example, the cellulolytic activity of the hyphomycete *Triscelophorous monosporus*, as expressed in the media, would lead one to conclude it is only moderately effective at producing these enzymes compared to the other organisms. However, analysis of the bound activity places more importance on this species relative to the others, and it may be a more important player in decomposition of cellulose than would be concluded if the media was only examined. Butler and Suberkropp (1986) found that even though *Heliscus lugdunensis* had high enzyme activity in culture it was less effective at causing weight loss and softening on oak leaves. They suggested that this may be due to differences in specificity or constituents masking the natural substrate that the fungus is unable to degrade. As a result hyphae cannot penetrate the leaf matrix and rather than releasing enzymes at critical points within the leaf they are released into the culture fluid.

Results from this study indicate that the species of aquatic oomycetes in question were able to produce enzymes associated with leaf decomposition and in some cases exuded levels comparable to the aquatic hyphomycetes in this study. However, in some instances the enzyme was released into the media and relatively small amounts were associated with the leaf itself, implying the fungi was unable to penetrate the leaf matrix or did so slowly. This might suggest an important role for these fungi during the later stages of decomposition. In general, most oomycetes in this study produced less enzyme activity for each of the decomposition related enzymes assayed. This might indicate that these species would be inferior competitors with hyphomycetes during decomposition. Future research avenues of interest would include an extensive examination of all enzymes produced by these fungi during decomposition, a comparison of enzyme production at different pH's and temperatures and also a determination of the competitive or synergistic interaction of the fungal species.

CONCLUSION

In summary, results of this study reveal a wide distribution of fungi within a stream. A survey of the fungi within the Ford River reveals the oomycetes, like the hyphomycetes, are prevalent in a diversity of habitats and may have a distinct successional pattern. In order to fully understand the habitat selection by fungal species, all areas of the stream, ranging from riffles to pools, should be investigated. Also, it was evident that temperature played a major role in the occurrence of the fungal species. However, the season during which the peak number of species was apparent differed between the two orders investigated in this study, indicating the importance of extensive studies that cover all the seasons throughout the year. Other orders of fungi not typically studied may be contributing to the decomposition process. Further investigation of spore numbers or hyphal mass and the relationship between the distribution of the oomycete species versus the hyphomycete species would be of interest.

In this study, the organisms were grown on leaf discs in stationary media flasks and were capable of producing enzymes involved in the degradation of plant material. Which indicates that these organisms are actively decomposing leaves in the pools as well as the riffles. While it was not possible to examine the entire array of enzymes produced by each organism several enzymes considered essential to the decomposition process of plant material were focused upon. In most instances there was more enzyme exuded into the media than retained on the leaf. If this same event is occurring in nature it would seem to indicate that much of the energy put into producing these enzymes is being lost to its surroundings or used for purposes other than leaf decomposition, possibly insect degradation.

While enzyme production for the species studied within the oomycete order was less than that of the hyphomycetes studied it was evident that these species were capable of producing the enzymes pertinent to degradation of leaf detritus and should be considered during microbial investigations of decomposition. Although some research has focused on enzymes produced by oomycete species for the degradation of a variety of pure substrates (Thompstone, 1985 ; Unestam, 1966) more research is needed that incorporates the actual substrates from nature as has been done with the hyphomycete species. Also, more attention needs to be focused on the interaction of fungal organisms at the family, order, and class level during the decomposition of plant material.

APPENDIX ONE

Table A-1 List of Hyphomycete and Oomycete Species Observed During the1990 and 1991 Field Seasons. Presence = +, Absence = o.

Species Observed	1990	1991
Hyphomycetes		
Alatospora acuminata Ingold	+	+
Anguillospora crassa Ingold	o	+
Anguillospora longissima (de Wildemann) Ingold	+	+
Articulospora grandis Greathead	+	+
Articulospora tetracladia Ingold	+	+
Campospora pellucidum (Grove) Hughes	o	+
Centrospora acerina (Hartig) Newhall	+	+
Centrospora aquatica Iqbal	o	+
Clavariopsis aquatica de Wildemann	+	+
Clavariopsis tentacula (Umphlett) Nilsson	o	+
Flagellospora curvula Ingold	+	+
Flagellospora penicilliodes Ingold	o	+
Heliscus lugdunensis Sacc. and Therry	+	o
Lemonniera aquatica de Wildemann	o	+
Lemonniera terretris Tubaki	o	+
Lunulospora curvula Ingold	+	+
Tetrachaetum elegans Ingold	o	+
Tetracladium marchalianum de Wildemann	+	+
Tetracladium setigerum (Grove) Ingold	o	+
Tricladium angulatum Ingold	+	+
Tricladium giganteum Ingold	o	+
Triscelophorus monosporus Ingold	+	+
Oomycetes		
Achlya americana de Bary	+	+
Achlya bisexualis Coker and A. Couch	+	+
Achlya caroliniana Coker	+	o
Achlya colorata Pringsheim	o	+
Achlya conspicua Coker	o	+
Achlya flagellata Coker	+	o
Achlya hypogyna Coker and Pemberton	+	+
Achlya klebsiana Pieters	+	o
Achlya polyandra Hildebrand	+	+
Achlya prolifera C. G. Nees	o	+
Achlya proliferoides Coker	o	+
Achlya racemosa Hildebrand	+	+
Dictyuchus monosporus Leitgeb	+	+
Saprolegnia australis	o	+
Saprolegnia diclina Humphrey	+	+
Saprolegnia ferax (Gruith) Thuret	+	+
Saprolegnia glomerata	o	+
Saprolegnia hypogyna (Pringsheim) de Bary	o	+
Saprolegnia litoralis Coker	o	+
Saprolegnia parasitica Coker	+	+
Saprolegnia terrestris	+	+

Table A-2 List of Hyphomycete and Oomycete species observed and percent frequency of occurrence at FEX Pool and Riffle sites from July 29 to September 10, 1990. Frequency based on 5 leaves per date.

	3days		7days		14days		21days		28days		56days	
Species observed	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool
Hyphomycetes												
Alatospora acuminata	0	0	0	0	0	0	0	0	0	0	0	0
Anguillospora longissima	0	0	0	0	0	0	0	0	0	0	0	0
Articulospora grandis	0	0	0	0	0	0	0	0	0	0	0	0
Articulospora tetracladia	20	0	0	0	0	0	20	40	20	0	0	20
Centrospora acerina	20	0	0	0	0	0	0	0	0	0	0	0
Clavariopsis aquatica	0	0	0	0	0	0	0	0	0	0	0	0
Flagellospora curvula	20	0	0	0	0	20	20	0	0	20	0	0
Heliscus lugdunensis	0	0	0	0	0	0	0	0	0	0	0	0
Lunulospora curvula	20	0	0	0	20	20	80	60	20	20	0	0
Tetracladium marchalianum	20	0	0	0	0	0	0	0	0	60	0	0
Tricladium angulatum	0	0	0	0	0	0	0	0	0	0	0	0
Triscelophorus monosporus	20	40	0	0	40	0	60	40	0	20	20	0
Oomycetes												
Achlya americana	20	0	0	0	0	0	0	40	0	0	0	0
Achlya bisexualis	40	40	80	100	20	60	40	40	0	0	0	0
Achlya caroliana	0	0	0	0	0	0	0	20	0	0	0	0
Achlya conspicua	0	20	0	0	0	0	0	0	0	0	0	20
Achlya flagellata	0	0	0	0	0	0	0	0	0	0	0	0
Achlya hypogyna	0	0	0	0	20	0	0	0	0	0	0	0
Achlya klebsiana	0	0	0	0	0	60	0	20	0	0	0	0
Achlya polyandra	0	60	0	0	0	0	0	0	0	0	0	0
Achlya racemosa	0	40	0	0	0	0	0	0	0	0	0	0
Dictyuchus monosporus	0	0	0	0	0	0	20	20	0	0	60	60
Saprolegnia diclina	0	0	0	0	0	0	0	0	0	0	0	0
Saprolegnia ferax	60	20	40	20	40	0	40	40	0	40	20	20
Saprolegnia parasitica	0	0	0	0	0	0	0	0	0	0	0	40
Saprolegnia terrestris	20	0	0	0	0	0	0	0	0	0	0	0

Table A-3 List of Hyphomycete and Oomycete species observed and percent frequency of occurrence at FCD Pool and Riffle sites from July 29 to September 10, 1990. Frequency based on 5 leaves per date.

	3days		7days		14days		21days		28days		56days	
Species observed	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool
Hyphomycetes												
Alatospora acuminata	0	0	0	0	0	0	0	0	0	0	0	0
Anguillospora longissima	0	0	0	0	0	0	0	0	0	0	0	0
Articulospora grandis	0	0	0	0	0	0	0	0	0	0	0	0
Articulospora tetracladia	20	40	0	0	0	0	20	40	20	0	0	20
Centrospora acerina	0	0	0	0	0	0	0	0	0	0	0	0
Clavariopsis aquatica	0	0	0	0	0	20	0	0	0	0	0	0
Flagellospora curvula	0	20	20	0	0	20	0	0	0	20	0	0
Heliscus lugdunensis	0	0	0	0	0	0	0	20	0	0	0	0
Lunulospora curvula	0	20	20	20	20	20	60	60	0	20	0	0
Tetracladium marchalianum	20	0	0	0	40	40	20	0	20	60	20	0
Tricladium angulatum	0	0	0	0	0	0	0	0	0	0	0	0
Triscelophorus monosporus	80	40	0	0	60	40	80	40	20	20	0	0
Oomycetes												
Achlya americana	0	0	0	0	0	0	0	0	0	0	0	0
Achlya bisexualis	40	40	0	0	20	20	60	60	0	20	0	0
Achlya caroliana	0	0	0	0	0	0	0	0	0	0	0	0
Achlya conspicua	0	0	0	0	0	0	0	0	0	0	0	0
Achlya flagellata	0	20	0	0	0	0	0	0	0	0	0	0
Achlya hypogyna	0	0	0	0	0	0	0	0	0	0	0	0
Achlya klebsiana	0	0	0	0	0	0	0	0	0	0	0	0
Achlya polyandra	0	0	0	0	0	0	0	0	0	0	0	0
Achlya racemosa	0	0	0	0	0	0	0	0	0	0	0	0
Dictyuchus monosporus	0	0	0	0	0	0	0	0	0	0	0	20
Saprolegnia diclina	0	0	0	0	0	0	0	0	0	0	0	20
Saprolegnia ferax	60	80	20	80	60	20	40	20	40	0	20	0
Saprolegnia parasitica	0	0	0	0	0	0	0	0	0	0	20	20
Saprolegnia terrestris	0	0	0	0	0	0	0	0	0	0	0	0

Table A-4 List of Hyphomycete and Oomycete species observed and percent frequency of occurrence at FEX and FCD Pool and Riffle sites from September 9 to December 10, 1990. Frequency based on 3 or 4 leaves per date.

	FEX						FCD					
	27days		48days		91days		27days		48days		91days	
Species observed	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool
Hyphomycetes												
Alatospora acuminata	0	0	0	0	0	25	0	0	0	0	25	50
Anguillospora longissima	0	33	33	0	100	75	33	0	33	0	100	100
Articulospora grandis	0	0	0	0	0	0	0	0	0	0	0	25
Articulospora tetracladia	0	0	33	0	0	0	0	0	0	0	0	0
Centrospora acerina	0	33	0	0	0	0	0	0	0	0	25	0
Clavariopsis aquatica	0	0	0	0	25	25	0	0	0	0	25	50
Flagellospora curvula	0	33	33	0	0	0	0	0	33	33	0	25
Heliscus lugdunensis	0	33	0	33	0	0	33	0	0	0	0	0
Lunulospora curvula	0	0	0	0	25	0	0	0	0	66	0	0
Tetracladium marchalianum	33	0	66	0	100	100	0	0	0	0	100	100
Tricladium angulatum	33	0	0	0	50	75	0	0	0	0	50	25
Triscelophorus monosporus	33	0	66	33	50	50	66	0	33	66	50	25
Oomycetes												
Achlya americana	0	0	0	0	25	0	0	0	0	0	0	0
Achlya bisexualis	0	0	0	33	25	75	0	0	0	33	0	50
Achlya caroliana	0	0	0	0	0	25	0	0	0	0	0	0
Achlya conspicua	0	0	0	0	0	0	0	0	0	0	0	0
Achlya flagellata	0	0	0	0	0	0	0	0	0	0	0	0
Achlya hypogyna	0	0	0	0	0	0	0	0	0	0	0	0
Achlya klebsiana	0	0	0	0	0	0	0	0	0	0	0	25
Achlya polyandra	0	0	0	0	0	0	0	0	0	0	0	0
Achlya racemosa	0	0	0	0	0	0	0	0	0	0	0	0
Dictyuchus monosporus	0	0	0	33	0	0	0	0	0	0	25	25
Saprolegnia diclina	0	0	0	0	25	0	0	0	0	0	0	0
Saprolegnia ferax	0	0	0	33	25	25	0	0	66	0	0	0
Saprolegnia parasitica	0	0	0	0	0	0	0	0	0	0	0	25
Saprolegnia terrestris	0	0	0	0	0	0	0	0	0	0	0	0

Table A-5 List of Hyphomycete species observed and percent frequency of occurrence at FEX and FCD sites from July 12 to August 19, 1991. Frequency based on 3 leaves per date and 3 discs per leaf.

	3days		7days		14days		18days		21days		22days	
Species observed	FEX	FCD	FEX	FCD	FEX	FCD	FEX	FCD	FEX	FCD	FEX	FCD
Alatospora acuminata	0	0	11	11	78	67	56	56	22	33	33	67
Anguillospora crassa	0	0	0	0	0	0	0	0	0	0	0	0
Anguillospora longissima	0	0	0	44	11	0	11	22	45	11	0	17
Articulospora grandis	0	0	0	0	0	0	0	0	0	0	0	0
Articulospora tetracladia	0	0	0	11	0	0	0	0	0	0	11	0
Campospora pelludium	0	0	0	0	0	0	0	0	0	0	0	0
Centrospora acerina	0	11	11	0	0	0	0	0	0	0	0	0
Centrospora aquatica	0	0	0	22	11	0	0	0	0	0	0	0
Clavariopsis aquatica	0	0	11	11	0	0	33	22	11	0	0	33
Clavariopsis tentacula	0	0	11	33	0	0	0	0	0	0	0	0
Flagellospora curvula	11	0	0	11	0	0	11	33	11	11	0	0
Flagellospora penicilliodes	0	0	0	0	0	0	0	11	0	0	0	0
Lemmonierra aquatica	11	0	0	22	11	0	0	0	0	0	0	0
Lemmonierra terretris	0	22	0	0	0	0	0	0	0	0	0	0
Lunulospora curvula	89	100	100	100	45	44	33	56	22	33	67	50
Tetracladium elegans	0	0	0	45	0	0	0	0	0	0	0	0
Tetracladium marchalianum	44	44	78	56	100	78	44	67	45	67	33	33
Tetracladium setigerum	0	0	0	22	0	0	0	0	0	0	0	0
Tricladium angulatum	0	0	0	11	11	11	11	0	11	0	0	0
Triscelophorus monosporus	22	44	33	100	22	44	67	33	56	11	67	67

Table A-6 List of Oomycete species observed and percent frequency of occurrence at FEX and FCD sites from July 12 to August 19, 1991. Frequency based on 3 leaves per date and 3 discs per leaf.

	3days		7days		14days		18days		21days		22days	
Species observed	FEX	FCD	FEX	FCD	FEX	FCD	FEX	FCD	FEX	FCD	FEX	FCD
Achlya americana	11	11	0	11	22	11	11	0	0	0	0	33
Achlya bisexualis	0	22	0	22	22	56	0	0	11	22	0	17
Achlya colorata	0	0	0	0	0	0	0	0	0	0	0	0
Achlya hypogyna	0	11	0	0	0	0	0	0	0	0	0	0
Achlya polyandra	0	0	0	0	33	33	0	0	0	0	0	0
Achlya prolifera	0	0	0	0	0	0	0	0	0	0	0	0
Achlya proliferoides	0	0	0	0	11	0	0	0	0	0	0	0
Achlya racemosa	0	0	0	0	11	0	0	0	11	22	0	0
Dictyuchus monosporus	0	0	0	11	11	0	0	11	0	0	33	0
Saprolegnia australis	0	0	0	0	0	11	0	0	0	11	0	0
Saprolegnia diclina	11	0	0	0	0	0	0	0	22	11	17	17
Saprolegnia ferax	56	45	0	44	56	56	33	44	22	22	100	83
Saprolegnia glomerata	0	0	0	0	11	0	0	0	0	0	0	0
Saprolegnia hypogyna	11	0	0	0	0	0	0	0	0	0	0	0
Saprolegnia litoralis	0	0	0	0	11	11	0	0	0	0	33	0
Saprolegnia parasitica	0	11	0	0	11	0	11	22	11	0	0	17
Saprolegnia terrestris	0	0	0	0	0	0	11	0	0	0	0	0

Table A-7 List of Hyphomycete species observed and percent frequency of occurrence at FEX and FCD sites from August 16 to September 13, 1991. Frequency based on 3 leaves per date and 3 discs per leaf.

Species observed	3days		7days		14days		21days		28days	
	FEX	FCD	FEX	FCD	FEX	FCD	FEX	FCD	FEX	FCD
Alatospora acuminata	22	0	11	0	56	67	33	22	45	11
Anguillospora crassa	11	0	0	0	0	0	0	0	0	0
Anguillospora longissima	22	22	22	11	33	22	0	0	67	67
Articulospora grandis	0	0	11	0	0	0	0	0	0	0
Articulospora tetracladia	11	0	0	0	11	0	0	0	0	0
Campospora pelludium	0	0	0	0	11	0	0	0	11	11
Centrospora acerina	0	11	0	0	0	0	0	0	0	0
Centrospora aquatica	0	0	0	0	0	0	0	33	0	0
Clavariopsis aquatica	0	0	0	0	0	0	11	0	44	33
Clavariopsis tentacula	0	0	0	0	0	0	0	0	0	0
Flagellospora curvula	0	0	0	0	11	0	0	0	0	11
Flagellospora penicilliodes	0	0	0	0	0	0	0	0	0	0
Lemmonierra aquatica	0	0	0	0	0	0	0	0	33	0
Lemmonierra terretris	0	0	0	0	0	0	0	0	0	0
Lunulospora curvula	78	89	67	89	44	22	11	22	44	11
Tetracladium elegans	0	0	0	0	0	0	0	0	0	0
Tetracladium marchalianum	67	67	100	100	55	22	55	100	78	67
Tetracladium setigerum	0	0	0	0	0	33	0	0	0	0
Tricladium angulatum	0	0	11	22	0	22	22	0	0	22
Triscelophorus monosporus	22	44	22	11	78	44	33	11	56	78

Table A-8 List of Oomycete species observed and percent frequency of occurrence at FEX and FCD sites from August 16 to September 13, 1991. Frequency based on 3 leaves per date and 3 discs per leaf.

	3days		7days		14days		21days		28days	
Species observed	FEX	FCD	FEX	FCD	FEX	FCD	FEX	FCD	FEX	FCD
Achlya americana	0	0	0	0	0	0	0	0	0	0
Achlya bisexualis	22	44	11	11	0	11	11	0	11	0
Achlya colorata	0	0	11	0	0	0	0	0	0	0
Achlya hypogyna	0	0	0	0	0	0	0	0	0	0
Achlya polyandra	0	0	0	0	0	0	0	0	0	0
Achlya prolifera	11	0	0	0	0	0	0	0	0	0
Achlya proliferoides	0	0	0	0	0	0	0	0	11	0
Achlya racemosa	11	22	0	0	0	0	0	0	0	0
Dictyuchus monosporus	0	0	0	0	0	0	0	11	0	11
Saprolegnia australis	11	0	11	0	0	0	0	0	0	0
Saprolegnia diclina	0	0	11	11	0	11	0	11	0	0
Saprolegnia ferax	22	0	33	45	0	11	44	11	0	0
Saprolegnia glomerata	0	0	0	0	0	0	0	0	0	0
Saprolegnia hypogyna	0	0	0	0	0	0	0	0	0	0
Saprolegnia litoralis	0	0	0	0	0	0	11	0	0	0
Saprolegnia parasitica	11	0	11	0	0	0	11	22	0	11
Saprolegnia terrestris	0	0	0	0	0	0	0	0	0	0

Table A-9 Nutrient parameter averages at FEX and FCD sites on the Ford River. Values are Means ± S.E.

Sampling Period	Site	NH4-N (ug/l)	NO3-N (ug/l)	NO2-N (ug/l)	SRP (ug/l)
July 29-Sept 10, 1990 (n=11)	FEX	19.1±1.8	14.7±1.7	2.9±0.4	2.7±0.3
	FCD	19.6±2.1	13.7±0.9	2.6±0.1	2.7±0.3
Sept 9-Oct 29, 1990 (n=15)	FEX	23.8±3.3	15.4±3.6	6.3±0.7	4.3±0.7
	FCD	22.9±3.0	14.2±3.6	6.7±0.6	4.4±1.0
Oct 29-Dec 10, 1990 (n=2)	FEX	48.0±0.0	80.2±46.9	8.9±0.9	8.9±4.3
	FCD	52.3±1.3	71.9±46.1	8.0±0.0	11.6±6.0
July 12-Aug 19, 1991 (n=12)	FEX	17.5±1.2	21.1±4.5	4.7±0.2	5.9±0.3
	FCD	16.5±1.2	17.4±2.8	4.4±0.2	5.6±0.3
Aug 16-Sept 13, 1991 (n=8)	FEX	13.1±1.1	8.9±1.4	4.3±0.3	4.8±0.1
	FCD	12.2±0.4	8.5±1.6	4.3±0.3	4.8±0.1

Table A-10 Physical and chemical parameter averages at FEX and FCD sites on the Ford River. Values are Mean ± S.E.

Sampling Period	Site	pH	Alkalinity	Hardness	Diss O2 (mg/l)	CL (mg/l)
July 29-Sept 10, 1990	FEX	8.1 ± 0.0	158 ± 3	179 ± 2	9.0 ± 0.1	3.3 ± 0.3
	FCD	8.1 ± 0.0	157 ± 3	180 ± 2	9.0 ± 0.1	3.3 ± 0.3
Sept 9-Oct 29, 1990	FEX	7.9 ± 0.0	131 ± 7	155 ± 7	10.8 ± 0.3	5.1 ± 0.3
	FCD	7.9 ± 0.0	134 ± 7	157 ± 7	10.8 ± 0.3	4.7 ± 0.3
Oct 29-Dec 10, 1990	FEX	7.7 ± 0.2	118 ± 9	150 ± 18	13.0 ± 0.2	4.1 ± 0.1
	FCD	7.7 ± 0.1	116 ± 5	153 ± 18	12.7 ± 0.1	3.6 ± 0.0
July 12-Aug 19, 1991	FEX	8.0 ± 0.0	153 ± 5	170 ± 3	8.6 ± 0.2	3.4 ± 0.1
	FCD	8.0 ± 0.0	152 ± 5	170 ± 3	8.5 ± 0.2	3.0 ± 0.2
Aug 16-Sept 13, 1991	FEX	8.1 ± 0.0	162 ± 3	177 ± 3	8.5 ± 0.2	4.5 ± 0.5
	FCD	8.1 ± 0.0	161 ± 3	179 ± 3	8.5 ± 0.2	3.7 ± 0.4

Table A-10 (con't)

Sampling Period	Site	Conductivity (uhmos)	Turbidity	Water Temp (^oC)	Air Temp (^oC)	Discharge (m^3/sec)
July 29-Sept 10, 1990	FEX	235 ± 6	1.4 ± 0.1	16.3 ± 0.7	16.9 ± 0.8	0.52 ± 0.04
	FCD	250 ± 4	1.4 ± 0.1	16.6 ± 0.4	16.5 ± 0.9	0.57 ± 0.04
Sept 9-Oct 29, 1990	FEX	179 ± 10	0.9 ± 0.1	8.9 ± 1.2	10.1 ± 1.4	
	FCD	186 ± 14	1.0 ± 0.1	8.6 ± 1.3	9.1± 1.4	
Oct 29-Dec 10, 1990	FEX	151 ± 11	0.8 ± 0.1	1.8 ± 0.8	3.3 ± 2.8	
	FCD	148 ± 14	0.8 ± 0.1	2.0 ± 1.0	4.3 ± 3.8	
July 12-Aug 19, 1991	FEX	240 ± 5	1.7 ± 0.2	17.4 ± 0.9	19.8 ± 1.6	0.62 ± 0.06
	FCD	224 ± 10	1.7 ± 0.2	17.3 ± 0.7	19.2 ± 1.2	0.75 ± 0.07
Aug 16-Sept 13, 1991	FEX	247 ± 13	1.8 ± 0.1	18.1 ± 0.8	20.8 ± 1.9	0.49 ± 0.06
	FCD	267 ± 13	1.8 ± 0.2	18.3 ± 0.7	20.2 ± 1.6	0.58 ± 0.06

APPENDIX TWO

1. Mineral Salts Medium

0.80 g	Ammonium nitrate (NH4NO3)
0.11 g	Sodium chloride (NaCl)
0.41 g	Potassium diphosphate (KH2PO4)
0.52 g	Potassium phosphate (K2HPO4)
0.49 g	Magnesium sulfate (MgSO4*7H2O)
0.15 g	Calcium chloride (CaCl2*2H2O)
1.00 mg	thiamine
5.00 ug	biotin
182 mg	glutamate

The above compounds were combined with approximately 800 ml of distilled water and adjusted to a pH of 7. The solution was then diluted to a liter volume and 50 ml aliquots of the medium were added to 250 ml flasks. The flasks were stoppered with cotton plugs, covered with aluminum foil and autoclaved for 20 minutes. For mineral salts solid plates and test tube slants, 15 g of agar was added to 100 ml dH2O.

2. Reducing Sugar Assay Reagents

a. Copper Reagent A

24 gm	Na2CO3 (anhydrous)
12 gm	Rochelle salt
16 gm	NaHCO3
144 gm	Na2SO4 (anhydrous)

Dilute to 800 ml

Filter if necessary and store at room temperature

b. Copper Reagent B

$4\%\ CuSO_4 \cdot 5H_2O$

36 gm Na_2SO_4

1-2 drops conc H_2SO_4 per 100 ml

Dilute to 200 ml with dH_2O

c. Combined Copper Reagent

Combine 4 parts of Reagent A with 1 part Reagent B

d. Arsenomolybdate Reagent

25 gm ammonium molybdate, dissolve in 450 ml dH_2O

21 ml conc. H_2SO_4 added to above with mixing

3 gm $Na_2HAsO_4 \cdot 7H_2O$ dissolved in 25 ml H_2O then
added to above mixture

Reagent is mixed and stored in an incubator at 37°C for 24 to 48 hours or, if needed quickly, reagent may be heated to 55°C for 25 min.

LITERATURE CITED

LITERATURE CITED

Akridge, R. E. and R. D. Koehn. 1987. Amphibious hyphomycetes from the San Marcos River in Texas. *Mycologia.* 79(2):228-233.

Alexopoulos, C. L. 1962. Introductory Mycology, 2nd. edit. John Wiley and Sons, Inc. New York. 613 pp.

Alberts, V. A. et al. 1989. Extracellular enzyme activity of some Saprolegniales from a Florida estuary. *Mycologia* 81(3):460-463.

Almin, K. E. and K.-E. Eriksson. 1967. Enzymic degradation of polymers: I. Viscosimetric method for the determination of enzymic activity. *Biochim. Biophys. Acta.* 139:238-247.

Anderson, A. J. 1978. Extracellular enzymes produced by *Colletotrichum lindemuthianum* and *Helminthosporium maydis* during growth on isolated bean and corn cell walls. *Phytopathology.* 68:1585-1589.

Anderson, N. H. and J. R. Sedell. 1979. Detritus processing by macroinvertebrates in stream ecosystems. *Ann. Rev. Entomol.* 24:351-377.

Anon. 1977. Bio-Rad Laboratories Technical Bulletin 1051. Bio-Rad Protein Assay. Bio-Rad Laboratories.

Arsuffi, T. L. and K. Suberkropp. 1984. Leaf processing capabilities of aquatic hyphomycetes: interspecific differences and influence on shredder feeding preferences. *Oikos.* 42:144-154.

Arsuffi, T. L. and K. Suberkropp. 1986. Growth of two stream caddisflies (Trichoptera) on leaves colonized by different fungal species. *Journal of the North American Benthological Society.* 5(4)297-305.

Arsuffi, T. L. and K. Suberkropp. 1988. Effects of fungal mycelia and enzymatically degraded leaves on feeding and performance of caddisfly (Tricoptera) larvae. *Journal of the North American Benthological Society.* 7(3):205-211.

Arsuffi, T. L. and K. Suberkropp. 1989. Selective feeding by shredders on leaf-colonizing stream fungi: comparison of macroinvertebrate taxa. *Oecologia.* 79:30-37.

Ayers, W. A., G. C. Papavizas, and A. F. Diem. 1966. Polygalacturonate trans-eliminase and polygalacturonase production by *Rhizoctonia solani*. *Phytopathology.* 56:1006-1011.

Barksdale, A. W. 1962. Concerning the species, *Achlya bisexualis*. *Mycologia.* 54:704-712.

Barlocher, F. 1980. Leaf-eating aquatic invertebrates as competitors of aquatic hyphomycetes. *Oecologia* . 47:303-306.

Barlocher, F. 1982. On the ecology of Ingoldian fungi. *Bioscience.* 32(7):581-586.

Barlocher, F. 1987. Aquatic hyphomycete spora in 10 streams of New Brunswick and Nova Scotia. *Canadian Journal of Botany* . 65:76-79.

Barlocher, F. 1990. Factors that delay colonization of fresh alder leaves by aquatic hyphomycetes. *Archiv fur Hydrobiologie.* 119:249-255.

Barlocher, F. 1992. Effects of drying and freezing autumn leaves on leaching and colonization by aquatic hyphomycetes. *Freshwater Biology.* 28:1-7.

Barlocher, F. and B. Kendrick. 1973. Fungi in the diet of *Gammarus pseudolimnaeus* (Amphipoda). *Oikos.* 24:295-300.

Barlocher, F. and B. Kendrick. 1973. Fungi and food preferences of *Gammarus pseudolimnaeus. Archives of Hydrobiologia.* 72(4):501-516.

Barlocher, F. and B. Kendrick. 1974. Dynamics of the fungal population on leaves in a stream. *Journal of Ecology.* 62:761-791.

Barlocher, F. and B. Kendrick. 1975. Leaf-conditioning by microorganisms. *Oecologia.* 20:359-362.

Barlocher, F. and B. Kendrick. 1981. The role of aquatic hyphomycetes in the trophic structure of streams. In: Wicklow, D.T. and Carroll, G.C. (eds). The Fungal Community : its organization and role in the ecosystem. Marcel Dekker, New York, pp.743-760.

Barlocher, F. and P.D. Premdas. 1988. Effects of pentachlorophenol on aquatic hyphomycetes. *Mycologia.* 80(1):135-137.

Barlocher, F. and J. Rosset. 1981. Aquatic hyphomycete spora of two Black Forest and two Swiss Jura streams. *Transactions of the British Mycological Society.* 76(3):479-483.

Barlocher, F. and M. Schweizer. 1983. Effects of leaf size and decay rate on colonization by aquatic hyphomycetes. *Oikos.* 4:205-210.

Bateman, D. F. and R. L. Millar. 1966. Pectic enzymes in tissue degradation. *Annu. Rev. Phytopathol.* 4:119-146.

Bateman, D. F. and H. G. Bashan. 1976. Degradation of plant cell walls and membranes by microbial enzymes. In Physiological Plant Pathology, encyc. of plant phys. New Ser. Vol 4. Edited by R. Heitefuss and P.U. Williams. Springer Verlag, Berlin pp.316-355.

Beneke, E. S. 1948a. A new species of *Achlya* and of *Dictyuchus*. *Journ. Elisha Mitchell Sci. Soc.* 64:261-265.

Benfield, E. F. and J. R. Webster. 1985. Shredder abundance and leaf break down in an Appalacian mountain stream. *Freshwater Biology.* 15:113-120.

Bengtsson, G. 1983. Habitat selection in two species of aquatic hyphomycetes. *Microbial Ecology.* 9:15-26.

Benke, A. C. et al. 1988. Bioenergetic considerations in the analysis of stream ecosystems. *Journal of the North American Benthological Society.* 7(4) 480-502.

Berner, K. E. and E. S. Chapman. 1977. The cellulolytic activity of six oomycetes. *Mycologia.* 69:1232-1236.

Bernfield, P.1955. Amylases, alpha and beta. Methods in Enzymology. Vol1:149-150.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry.* 72:248-254.

Butler, S. K. and K.Suberkropp. 1986. Aquatic hyphomycetes on oak leaves: Comparison of growth, degradation and palatability. *Mycologia.* 78(6):922-928.

Butty, A-KN, F. T. Mhaiser, and N. M. Ali. 1989. Isolation of three water molds from two freshwater fishes and insect exuviae. *J. Environ. Sci. Health,* Part A. A24(1):17-22.

Cantino and Turian. 1959. Physiology and development of lower fungi (Phycomycetes). *Annu. Rev. Microbiol.* 13:97-124.

Chamier, A.-C. and P. A. Dixon. 1982. Pectinases in leaf degradation by aquatic hyphomycetes. I: the field study. *Oecologia.* 52:109-115.

Chamier, A.-C. and P. A. Dixon. 1982. Pectinases in leaf degradation by aquatic hyphomycetes: the enzymes and leaf maceration. *Journal of General Microbiology* . 128:2469-2483.

Chamier, A.-C., P. A. Dixon and S. A. Archer. 1984. The spatial distribution of fungi on decomposing alder leaves in a freshwater stream. *Oecologia*. 64:92-103.

Chamier, A.-C. 1985. Cell-wall-degrading enzymes of aquatic hyphomycetes: a review. *Botanical Journal of the Linnean Society*. 91:67-81.

Chamier, A.-C. and L. G. Willoughby. 1986. The role of fungi in the diet of *Gammarus pulex* (L.): an enzymatic study. *Freshwater Biology*. 16:197-208.

Chamier, A.-C. 1987. Effect of pH on microbial degradation of leaf litter in seven streams of the English Lake District. *Oecologia*. 71:491-500.

Chandrashekar, K. R., K. R. Sridhar and K. M. Kaveriappa. 1989. The palatability of rubber leaves colonized by aquatic hyphomycetes. *Archives of Hydrobiologia*. 151(3):361-369.

Chauvet, E. 1987. Changes in the chemical composition of alder, poplar, and willow leaves during decomposition in a river. *Hydrobiologia*. 148:35-44.

Chergui, H. and E. Pattee. 1988. The dynamics of hyphomycetes on decaying leaves in the network of the River Rhone (Fr ance). *Archives of Hydrobiologia*. 114(1):3-20.

Coker, W. C. 1923. The Saprolegniaceae, with notes on other water molds. 201 pp. Univ. North Carolina Press, Chapel Hill.

Coker, W. C. and V. D. Matthews. 1937. Saprolegniales. *North American Flora*. 2(1):15-67.

Cooke, W.B. 1961. Pollution effects on the fungus population of a stream. *Ecology*. 42:1-18.

Covich, A. P. 1988. Geographical and historical comparisons of neotropical streams: biotic diversity and detrital processing in highly variable environments. *Journal of the North American Benthological Society*. 7:361-386.

Dayal, R. and R. N. Tandon. 1962. Ecological studies of some aquatic Phycomycetes. I. *Hydrobiologia*. 20:121-127.

Dayal, R. and R. N. Tandon. 1963. Ecological studies of some aquatic Phycomycetes. II. Fungi in relation to chemical factors of the water. *Hydrobiologia*. 22:324-330.

Deacon, J. W. 1979. Cellulose decomposition by Pythium spp. and other fungi. *Transactions of the British Mycological Society*. 72:469-477.

Descals, E., J. Webster and B. S. Dyko. 1977. Taxonomic studies on aquatic hyphomycetes. I. *Lemonniera* de Wildeman. *Transactions of the British Mycological Society.* 69(1)89-109.

Dick, M. W. 1966. The Saprolegniaceae of the environs of Blelham Tarn : sampling techniques and estimation of propagule numbers. *Journal of General Microbiology.* 42:257-282.

Dick, M. W. 1969. Morphology and taxonomy of the Oomycetes, with special reference to Saprolegniaceae, Leptomitaceae and Pythiaceae. I. Sexual Reproduction. *New Phytology.* 68:751-775.

Dick, M. W. 1972. Morphology and taxonomy of the Oomycetes, with special reference to Saprolegniaceae, Leptomitaceae and Pythiaceae. II. Cytogenetic Systems. *New Phytology.* 71:1151-1159.

Dick, M. W. and H. V. Newby. 1961. The occurrence and distribution of Saprolegniaceae in certain soils of South-East England. I. Occurrence. *Journal of Ecology.* 49:403-419.

Eriksson, K.-E. 1978. In: Biotechnology and Bioengineering, vol. XX : Enzyme mechanisms involved in cellular hydrolysis by the rot fungus *Sporotrichum pulverulentum.* John Wiley, pp.317-332.

Findlay, S. E. G., J. L. Meyer and P. J. Smith. 1986. Incorporation of microbial biomass by *Peltoperla* sp. (Plecoptera) and *Tipula* sp. (Diptera). *Journal of the North American Benthological Society.* 5(4):306-310.

Findlay, S. E. G. and T. L. Arsuffi. 1989. Microbial growth and detritus transformations during decomposition of leaf litter in a stream. *Freshwater Biology.* 21:261-269.

Forbes, E. J. 1935. Water moulds of the Manchester district. *Mem. Proc. Manchester Lit. phil. Soc.* 79:1-11.

Fuller, M. S. and R. O. Poyton. 1962. A new technique for the isolation of aquatic fungi. *Bioscience.* 14(9):45-46.

Gessner, M. O. and J. Schwoerbel. 1989. Leaching kinetics of fresh leaf-litter with implications for the current concept of leaf processing in streams. *Archiv fur Hydrobiologie.* 115:81-90.

Gleason, F. H., C. R. Rudolph, and J. S. Price. 1970a. Growth of certain aquatic oomycetes on amino acids. I. *Saprolegnia, Achlya, Leptolegnia,* and *Dictyuchus. Physiologia.* 23:513-516.

Gleason, F. H. 1973. Uptake of amino acids by Saprolegniales. *Mycologia.* 65:465-468.

Goksoyr, J. and J. Eriksen. 1980. Cellulases. In : Economic Microbiology (Ed. A.H. Rose), vol. 5. Microbial Enzymes and Bioconversions, pp. 283-330. Academic Press, London.

Golladay, S. W. and R. L. Sinsabaugh. 1991. Biofilm development on leaf and wood surfaces in a boreal river. *Freshwater Biology*. 25:437-450.

Gupta, A. K. and R. S. Mehrotra. 1989. Seasonal periodicity of aquatic fungi tanks at Kurukshetra, India. *Hydrobiologia*. 173(3):219-229.

Hallett, I. C and M. W. Dick. 1981. Seasonal and diurnal fluctuations of oomycete propagule numbers in the free water of a fresh water lake. *Journal of Ecology*. 69(2):671-692.

Hankin, L. and S. L. Anagnostakis. 1975. The use of solid media for the detection of enzyme production by fungi. *Mycologia*. 67:597-607.

Harris, D. C. 1986. Methods for preparing, estimating and diluting suspensions of *Phytophthora cactorum* zoospores. *Transactions of the British Mycological Society*. 86(3):482-486.

Hasija, S. K. and S. Batra. 1978. The distribution of *Achlya americana* (Saprolegniales) in different aquatic habitats at Jabalpur, India. *Hydrobiologia*. 61(3):277-279.

Hatai, K., L. G. Willoughby, and G. W. Beakes. 1990. Some characteristics of *Saprolegnia* obtained from fish hatcheries in Japan. *Mycological Research*. 94(2):182-190.

Herman, R. P. and M. M. Luchini. 1989. Lipoxygenase activity in the oomycete *Saprolegnia* is dependent on environmental cues and reproductive competence. *Experimental Mycology*. 13:372-379.

Howard, K. L. 1971. Oospore types in the Saprolegniaceae. *Mycologia*. 63:679.

Hughes, G. C. 1962. Seasonal periodicity of the Saprolegniaceae in the South-Eastern United States. *Transactions of the British Mycolological Society*. 45(4):519-531.

Ingold, C. T. 1975. An Illustrated Guide to Aquatic and Water-Borne Hyphomycetes. Freshwater Biological Association. 96pp.

Ingold, C. T. 1979. Advances in the study of so-called aquatic hyphomycetes. *American Journal of Botany*. 66(2):218-226.

Iqbal, S. H. and J. Webster. 1973. The trapping of aquatic hyphomycete spores by air bubbles. *Transactions of the British Mycological Society*. 60(1):37-48.

Iqbal, S. H. and J. Webster. 1973. Aquatic hyphomycete spora of the River Exe and its tributaries. *Transactions of the British Mycological Society.* 61(2):331-346.

Iqbal, S. H. and J. Webster. 1979. Aquatic hyphomycete spora of some Dartmoor streams. *Transactions of the British Mycological Society.* 69(2):233-241.

Johnson, T. W. 1956. The genus Achlya: morphology and taxonomy. Univ. of Michigan Press, Ann Arbor. 180 pp.

Kaushik, N. K. and H. B. N. Hynes. 1971. The fate of dead leaves that fall into streams. *Archives fur Hydrobiologie.* 68:465-515.

Khulbe, R. D. 1980. Occurrence of water molds in relation to hydrogen ion concentration in some lakes of Nainital, India. *Hydrobiologia.* 69:3-5.

Khulbe, R. D. and K. S. Bhargava. 1977. Distribution and seasonal periodicity of water moulds in some lakes of Nainital Hills, India. *Hydrobiologia.* 54:67-72.

Kostalos, M. and R. L. Seymour. 1976. Role of microbial enriched detritus in the nutrition of *Gammarus minus* (Amphipoda). *Oikos.* 27:512-516.

Leff, L. G. and J. V. MacArthur. 1990. Effect of nutrient content on leaf decomposition in a coastal plain stream: A comparison of green and senescent leaves. *Journal of Freshwater Ecology.* 5(3):269-277.

Leone, G., E. A. M. Schoffelmeer, and J. Van den Heuvel. 1990. Purification and characterization of a constitutive polygalacturonase associated with the infection process of French bean leaves by *Botrytis cinerea. Canadian Journal of Botany.* 68:1921-1930.

Lund, A. 1934. Studies on Danish freshwater Phycomycetes and notes on their occurrence particularly relative to the Hydrogen-ion concentration of the water. *Kgl.Danske, Videnske. Selsk. Skrift. Natur. Math. Afd.* 9. 6:1-97.

MacArthur, J. V., L. G. Leff, D. A. Kovacic, and J. Jaroscak. 1986. Green leaf decomposition in coastal plain streams. *Journal of Freshwater Ecology.* 3:553-558.

MacKay, R. J. and J. Kalff. 1973. Ecology of two related species of caddisfly larvae in the organic substrates of a woodland stream. *Ecology.* 54:499-511.

Manoharachary, C. and P. Ramarao. 1981. Seasonal variation and distribution of fungi in two fresh water ponds of Andhra Pradesh, India. *Proc. Ind. Acad. Sci.* 90:237-243.

Marcus, J. H. and L. G. Willoughby. 1978. Fungi as food for the aquatic invertebrate *Ascellus aquaticus*. *Transactions of the British Mycological Society*. 70(1):143-146.

McIntyre, J. L. and L. Hankin. 1978. An examination of *Phytopthera* on solid and liquid media. *Canadian Journal of Microbiology*. 24:75-78.

Meyer, J. L. 1980. Dynamics of phosphorus and organic matter during leaf decomposition in a forest stream. *Oikos*. 34:44-53.

Meyer, J. L. et al. 1988. Elemental dynamics in streams. *Journal of the North American Benthological Society*. 7(4):410-432.

Mer, G. S., S. C. Sati and R. D. Khulbe. 1980. Occurrence, distribution and seasonal periodicity of some aquatic fungi of Sat-Tal (Nainital), India. *Hydrobiologia*. 76(3):201-205.

Miele, W. H. and A. E. Linkins. 1974. Cellulase activity during the growth of *Achlya bisexualis* on glucose, cellulose, and selected polysaccharides. *Canadian Journal of Botany*. 56: 1974-1981.

Milanez, A. I. 1966. A study of the aquatic Phycomycetes from the Gull Lake area in Michigan. PhD thesis. Michigan St. Univ. E.L. Michigan.

Miller, L. and J. D. MacMillan. 1970. Mode of action of pectic enzymes. II. Further purification of exopolygalacturonate lyase and pectinesterase from *Clostridium multifermentans*. *Journal of Bacteriology*. 102(1):72-78.

Monk, D. C. 1976. The distribution of cellulase in freshwater invertebrates of different feeding habits. *Freshwater Biology*. 6:471-475.

Mishra, R. C. and R. S. Dwivedi. 1986. Physico-chemical properties and their influence on the occurrence and seasonal periodicity of aquatic moulds in an alkaline lake. *Tropical Ecology*. 27(2):210-219.

Misra, J. K. 1982. Occurrence, distribution and seasonality of aquatic fungi as affected by chemical factors in six alkaline ponds of India. *Hydrobiologia*. 97:185-191.

Misra, J. K. 1983. Occurrence, distribution and seasonality of aquatic fungi as affected by water temperature in certain alkaline ponds of India. *Indian Journal of Plant Pathology*. 1:133-140.

Mitchell, R. T. and J. W. Deacon. 1986. Selective accumulation of zoospores of chytridiomycetes and oomycetes on cellulose and chitin. *Transactions of the British Mycological Society*. 86(2):219-223.

Novdes-Ledieu, M., A. Jimenez-Martinez, and J. R. Villanueva. 1967. Chemical composition of the hyphal walls of Phycomycetes. *Journal of General Microbiology.* 47:237-245.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry.* 153:375-380.

Nilsson, S. 1964. Freshwater hyphomycetes. *Symb. bot. upsal.* 18, 130pp.

Park, D. 1975. A celluloytic pythiaceous fungus. *Transactions of the British Mycological Society.* 65:249-257.

Park, D. 1980. A two-year study of numbers of cellulolytic *Pythium* in river water. *Transactions of the British Mycological Society.* 74:253-258.

Park, D. and W. McKee. 1978. Cellulolytic Phythium as a component of the river mycoflora. *Transactions of the British Mycological Society.* 71:251-259.

Parker, B. C., R. D. Preston, and G. E. Fogg. 1963. Studies of the structure and chemical composition of the cell walls of Vaucheriaceae and Saprolegniacea. *Proc. R. Soc. London Ser. B.* 158:435-447.

Perrott, P. E. 1960. The ecology of some aquatic Phycomycetes. *Transactions of the British Mycological Society.* 43:19-30.

Peterson, R. H. 1962. Aquatic hyphomycetes from North America. I. Aleuriosporae (part I), and key to the genera. *Mycologia.* 54:117-151.

Peterson, R. H. 1963a. Aquatic hyphomycetes from North America. II. Aleuriosporae (part II), and Blastosporae. *Mycologia.* 55:18-29.

Peterson, R. H. 1963b. Aquatic hyphomycetes from North America. III. Phialosporae and miscellaneous species. *Mycologia.* 55:570-581.

Peterson, R. H. and K. Cummins. 1974. Leaf processing in a woodland stream. *Freshwater Biology.* 343-368.

Ranzoni, R. V. 1951. Nutrient requirements for two species of aquatic hyphomycetes. *Mycologia.* 43:130-141.

Raper, J. R. 1937. A method of freeing fungi from bacterial contamination. *Science.* 85:342.

Reese, E. T. 1977. In : Loewus, F.A. and Runeckles, V.C. (eds.), Recent advances in phytochemistry, vol. II : The structure, biosynthesis, and degradation of wood. Plenum Press, NY, pp. 311-367.

Ried, J. L. and A. Collmer. 1985. Activity stain for rapid characterization of pectic enzymes in isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gels. *Applied and Environmental Microbiology.* 50(3):615-622.

Risley, L. S. and D. A. Crossley, Jr. 1988. Herbivore-caused greenfall in the southern Appalachians. *Ecology.* 69:1118-1127.

Roberts, R. E. 1963. A study of the distribution of certain members of the Saprolegniales. *Transactions of the British Mycological Society.* 46(2):213-224.

Roldan, A., E. Descals and M. Honrubia. 1989. Pure culture studies on *Tetracladium. Mycological Research.* 93(4)452-465.

Rossi, L. and A. E. Fano. 1979. Role of fungi in the trophic niche of the congeneric detritivorous *Asellus aquaticus* and *A. coxalis* (Isopoda). *Oikos.* 32:380-385.

Scott, W. W., J. R. Powell and R. L. Seymour. 1963. Pure culture techniques applied to the growth of *Saprolegnia* spp. on a chemically defined medium. *Virginia Journal of Science (N.S.)* 14(2):42-46.

Seymour, R. L. 1970. The Genus Saprolegnia. Verlag Von J. Cramer. 124pp.

Shearer, C. A. and L. C. Lane. 1983. Comparison of three techniques for the study of aquatic hyphomycete communities. *Mycologia.* 75(3):498-508.

Shearer, C. A. and J. Webster. 1985. Aquatic hyphomycete communities in the River Teign. I. Longitudinal distribution patterns. *Transactions of the British Mycological Society.* 84(3):489-501.

Shearer, C. A. and J. Webster. 1985. Aquatic hyphomycete communities in the River Teign. II. Temporal distribution patterns. *Transactions of the British Mycological Society.* 84(3):503-507.

Singh, N. 1982. Cellulase decomposition by some tropical aquatic hyphomycetes. *Transactions of the British Mycological Society.* 70:560-561.

Sinsabaugh, R. L., E. F. Benfield and A. E. Linkins. 1981. Cellulase activity associated with the decomposition of leaf litter in a woodland stream. *Oikos.* 36:184-190.

Sinsabaugh, R. L. and A. E. Linkins. 1990. Enzymic and chemical analysis of particulate organic matter from a boreal river. *Freshwater Biology.* 23:301-309.

Sinsabaugh, R. L., S. W. Golladay and A. E. Linkins. 1991. Comparison of epilithic and epixylic biofilm development in a boreal river. *Freshwater Biology*. 25:179-187.

Somogyi, M. 1952. Notes on sugar determination. *Journal of Biological Chemistry*. 195:19-23.

Sparling, G. P., B. G. Ord, and D. Vaughn. 1981. Changes in microbial biomass and activity in soils amended with phenolic acids. *Journal of Biological Biochemistry*. 13:455-460.

Sparrow, F. K., Jr. 1952d. Phycomycetes from the Douglas Lake region of Northern Michigan. *Mycologia*. 44:759-772.

Sparrow, F.K. 1960. Aquatic Phycomycetes. 2nd rev. edition.

Sridhar, K. R. and K. M. Kaveriappa. 1989. Colonization of leaves by water-borne hyphomycetes in a tropical stream. *Mycological Research*. 92(4):392-396.

Srivastava, G.C. 1967. Ecological studies on some aquatic fungi of Gorakhpur, India. *Hydrobiologia*. 30:385-404.

Stout, R.J., W. H. Taft, and R. W. Merritt. 1985. Patterns of macroinvertebrate colonization on fresh and senescent alder leaves in two Michigan streams. *Freshwater Biology*. 15:573-580.

Suberkropp, K. 1984. Effect of temperature on seasonal occurrence of aquatic hyphomycetes. *Transactions of the British Mycological Society*. 82(1):53-62.

Suberkropp, K. 1991. Relationships between growth and sporulation of aquatic hyphomycetes on decomposing leaf litter. *Mycological Research*. 95(7):843-850.

Suberkropp, K. and T. L. Arsuffi. 1984. Degradation, growth, and changes in palatability of leaves colonized by six aquatic hyphomycete species. *Mycologia*. 76(3):398-407.

Suberkropp, K. and M. J. Klug. 1976. Fungi and bacteria associated with leaves during processing in a woodland stream. *Ecology*. 57:707-719.

Suberkropp, K. and M. J. Klug. 1980. The maceration of deciduous leaf litter by aquatic hyphomycetes. *Canadian Journal of Botany*. 58:1025-1031.

Suberkropp, K. and M.J. Klug. 1980. Degradation of leaf litter by aquatic hyphomycetes. In : Wicklow, D.T. & G.C. Carroll (eds). The Fungal Community : its organization and role in the ecosystem. Marcel Dekker, New York, pp.761-776.

Suberkropp, K., G. L. Godshalk and M. J. Klug. 1976. Changes in the chemical composition of leaves during processing in a woodland stream. *Ecology.* 57:720-727.

Suberkropp, K., T. L. Arsuffi and J. P. Anderson. 1983. Comparison of degradative ability, enzymatic activity, and palatability of aquatic hyphomycetes grown on leaf litter. *Applied Environmental Microbiology.* 46:237-244.

Suryanarayana, K. and D. Des S. Thomas. 1986. Asexual spore release from saprolegniaceous water moulds: Involvement of calmodulin. *Journal of General Microbiology.* 132:593-598.

Suzuki, S. 1960. Ecological studies on the aquatic fungi in the Arakawa River. *Japanase Journal of Limnology.* 21:17-24.

Taylor, B. R., D. Parkinson, and W. F. J. Parsons. 1989. Nitrogen and lignin content as predictors of litter decay rates: A microcosm test. *Ecology.* 80:97-104.

Thomas, D. Des S. and J. T. Mullins. 1967. Role of enzymatic wall-softening in plant morphogenesis : hormonal induction in *Achlya. Science.* 156:84-85.

Thomas, D. Des S. and J. T. Mullins. 1969. Cellulase induction and wall extension in the water mold *Achlya ambisexualis. Physiol. Plant.* 22:347-353.

Thomas, K., G. A. Chilvers and R. H. Norris. 1989. Seasonal occurrence of conidia of aquatic hyphomycetes (fungi) in Lees Creek, Australian Capital Territory. *Australian Journal of Marine and Freshwater Research.* 40:11-23.

Thomas, K., G. A. Chilvers and R. H. Norris. 1992. Aquatic hyphomycetes from different substrates : Substrate preference and seasonal occurrence. *Australian Journal of Marine and Freshwater Research.* 43:491-509.

Thompson, P. L. and F. Barlocher. 1989. Effect of pH on leaf breakdown in streams and in the laboratory. *Journal of the North American Benthological Society.* 8(3):203-210.

Thompstone, A. and N. J. Dix. 1985. Cellulase activity in the Saprolegniaceae. *Transactions of the British Mycological Society.* 85(2):361-366.

Thornton, D. R. 1963. The physiology and nutrition of some aquatic hyphomycetes. *Journal of General Microbiology* 33:23-31.

Thornton, D. R. and M. H. Fox. 1968. The free amino acid pools of two aquatic hyphomycetes. *Experentia.* 24:393-394.

Torzilla, A. P. 1978. Isolation and partial characterization of pectic enzymes produced by *Blastocladia ramosa. Experimental Mycology.* 2:1-11.

Triska, F. S. 1970. Seasonal distribution of aquatic hyphomycetes in relation to the disappearance of leaf litter from a woodland stream. PhD thesis. U. of Pitts. Pitts PA.

Triska, F. S., J. R. Sedell and B. Buckley. 1975. The processing of conifer and hardwood leaves in two coniferous forest streams: II. Biochemical and nutrient changes. *Verh, Int. Verein. Limnol.* 19:1628-1639.

Tubaki, K. 1958. Studies on the Japanese hyphomycetes. V. Leaf and stem group with a discussion of the classification of Hyphomycetes and their perfect stages. *Journal of the Hattori Botanical Laboratory.* 20:221-228.

Unestam, T. 1966. Chitinolytic, cellulolytic, and pectinolytic activity *in vitro* of some parasitic and saprophytic oomycetes. *Physiologia Plantarum.* 49:15-30.

Valiela, I., L. Koumjian, T. Swain, J. M. Teal, and J. E. Hobbie. 1979. Cinnamic acid inhibition of detritus feeding. *Nature.* 280:55-57.

Van der Merwe, W. J. J. and W. J. Jooste. 1988. A synecological study of aquatic hyphomycetes in the Mooi River, Western Transvaal, and their significance in the decomposition of allocthonous leaf litter. *S. Afr. J. Sci./S.-Afr. Tydskr. Wet.* 84(5):314-320.

Van Kley, H. and S. M. Hale. 1977. Assay for protein by dye binding. *Analytical Biochemistry.* 81:485-487.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Science.* 37:130-137.

Waterhouse, G.M. 1942. Some water moulds of the Hogsmill River collected from 1937 to 1939. *Transactions of the British Mycological Society.* 25:315-325.

Webster, J. R. and E. F. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. *Ann. Rev. Ecol. Syst.* 17:567-594.

Webster, J. R. and E. Descals. 1981. Morphology, distribution, and ecology of conidial fungi in freshwater habitats. In *Biology of Conidial Fungi*, vol. 1 (ed. G.T. Cole & B. Kendrick), pp. 295-355. New York: Academic Press.

Webster, J. R., S. T. Moran and R. A. Davey. 1976. Growth and sporulation of *Tricladium chaetocladium* and *Lunulospora curvula* in relation to temperature. *Transactions of the British Mycological Society.* 67:491-495.

Webster, J. R. and T. H. Towfik. 1972. Sporulation of aquatic hyphomycetes in relation to aeration. *Transactions of the British Mycological Society.* 59: 353-364.

Willoughby, L. G. 1962. The occurrence and distribution of reproductive spores of Saprolegniales in fresh water. *Journal of Ecology.* 50:733-59.

Willoughby, L. G. 1965. Some observations on the location of sites of fungal activity at Blelham Tarn. *Hydrobiologia.* 25:352-356.

Willoughby, L. G. and V. G. Collins. 1966. A study of the distribution of fungal spores and bacteria in Blelham Tarn and its associated streams. *Nova Hedwigia.* 12:150-171.

Wolf, F. T. 1944. The aquatic Oomycetes of Wisconsin. Part. I. Univ. Wisconsin Press, Madison, Wisconsin. 64pp.

Wolf, F. T. and F. A. Wolf. 1941. Aquatic Phycomycetes from the Everglades region of Florida. *Lloydia.* 4:270-275.

Wood, W. A. and S. T. Kellogg. 1988. Methods in Enzymology: Cellulose and Hemicellulose. Vol 160. Academic Press, Inc. 774pp.

Wood, W. A. and S. T. Kellogg. 1988. Methods in Enzymology: Lignin, Pectin, and Chitin. Vol 161. Academic Press, Inc. 574pp.

Wood-Eggenschwiler,S. and F. Barlocher. 1983. Aquatic hyphomycetes in sixteen streams in France, Germany and Switzerland. *Transactions of the British Mycological Society.* 81(2):371-379.

Zare-Maiven, H. and C. A. Shearer. 1988. Extracellular enzyme production and cell wall degradation by freshwater lignicolous fungi. *Mycologia.* 80(3):365-375.

Zare-Maiven, H. and C. A. Shearer. 1988. Wood decay activity and cellulase production by freshwater lignicolous fungi. *Int. Biodeterior.* 24(6):459-474.

Ziegler, A. W. 1962. The seasonal periodicity of water molds. I. Field studies. *Mycopath. Mycol. Appl.* 89-92.