CHAPTER 15

Fungi: Biomass, Production, and Sporulation of Aquatic Hyphomycetes

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I. INTRODUCTION

Fungi are common inhabitants of stream ecosystems. All phyla of true fungi (Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota) and also the Oomycota (kingdom Stramenopila) that are morphologically similar to fungi can be observed in or isolated from stream environments. The most ecologically important and well studied fungi in streams are the "aquatic hyphomycetes," which are anamorphs (asexual stages) of ascomycetes or basidiomycetes. These fungi are capable of completing their entire asexual life cycle underwater starting from colonization of suitable substrate followed by intramatrical mycelial growth and abundant sporulation. Up to 80% of fungal production may be invested into conidia (i.e., asexual spores) (Suberkropp 1991). Conidia of aquatic hyphomycetes are often tetraradiate, variously branched, or filiform (rarely conventionally shaped) (Figure 15.1), which is an adaptation to dispersal in flowing water and enhances the probability of attachment to fresh substrates (Webster and Descals 1981).

Allochthonous organic matter (leaves of deciduous trees, twigs, etc.) is an important source of energy and nutrients in small forest streams (Fisher and Likens 1973, Kaushik and Hynes 1968) and fungi are the main colonizers of this plant litter. Maximum fungal biomass and sporulation rate of aquatic hyphomycetes correlate well with plant litter breakdown rate (Gessner and Chauvet 1994, Niyogi *et al.* 2003), suggesting that fungi are

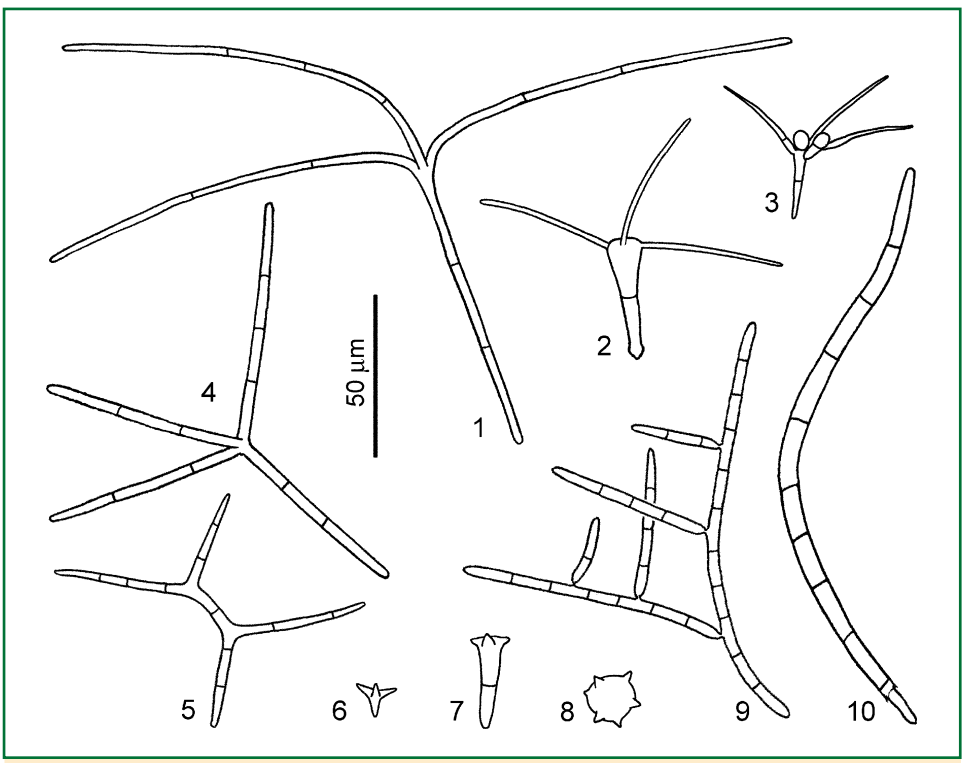

FIGURE 15.1 Conidia of aquatic hyphomycetes. 1. *Tetrachaetum elegans*. 2. *Clavariopsis aquatica*. 3. *Tetracladium marchalianum*. 4. *Lemonniera aquatica*. 5. *Tricladium angulatum*. 6. *Heliscella stellata*. 7. *Heliscus lugdunensis*. 8. *Goniopila monticola* (or *Margaritispora aquatica*). 9. *Varicosporium elodeae*. 10. *Anguillospora longissima*.

the primary decomposers of this organic matter in headwater streams. Fungal biomass accounts for 95 to >99% of total microbial biomass (fungi plus bacteria) associated with submerged decaying plant litter (Baldy and Gessner 1997, Gulis and Suberkropp 2003a, Hieber and Gessner 2002) and fungal production is also typically greater than that of bacteria (Baldy *et al.* 2002, Weyers and Suberkropp 1996). Annual fungal production on an areal basis in small streams can be comparable to or higher than invertebrate secondary production (Methvin and Suberkropp 2003, Suberkropp 1997). Fungi mineralize organic carbon of plant litter, convert it into their own biomass, which can account for up to 18–23% of total detrital mass (Methvin and Suberkropp 2003, Suberkropp 1995), macerate leaf litter, and release fine particulate organic matter (including conidia) and dissolved organic matter that become available to other stream dwellers. Fungal colonization results in "conditioning" of leaf litter that increases its nutritional value and palatability to shredding invertebrates. It is well documented that aquatic invertebrates prefer to feed and grow better on leaf litter colonized by fungi in comparison to uncolonized leaves (Bärlocher 1985, Suberkropp 2003). Therefore, apart from the decomposition of organic matter, aquatic hyphomycetes also mediate energy and nutrient transfer to higher trophic levels (Bärlocher and Kendrick 1976).

Despite their key importance in the functioning of stream ecosystems, aquatic fungi, historically, have received much less attention than macroinvertebrates, algae, fishes, or

even bacteria. Aquatic hyphomycetes were discovered as a distinctive ecological group only in the middle of the last century (Ingold 1942). This may be explained in part by the microscopic size and habitat of these organisms (inside opaque submerged decaying substrates). Stream ecologists recognized the importance of fungi about three decades ago (Bärlocher and Kendrick 1974, Kaushik and Hynes 1968, Suberkropp and Klug 1976). Several useful reviews summarizing achievements in the field have been published since then (Bärlocher 1992, Gessner *et al.* 2003, Gessner and Newell 2002, Gessner *et al.* 1997, Suberkropp 1992).

This chapter focuses on quantitative methods to study the ecology of aquatic fungi. Specific objectives are to (1) describe techniques to determine concentration of conidia and community structure of aquatic hyphomycetes in transport, (2) present approaches for estimating reproduction (sporulation rate) of aquatic hyphomycetes, and (3) introduce methods to estimate fungal biomass and production associated with decaying submerged plant litter.

II. GENERAL DESIGN

A. Site Selection and General Considerations

Submerged decaying plant litter that serves as substrate for aquatic fungi can be found in almost all types of lotic habitats. Alternatively, plant material introduced as leaf bags or packs can be used after appropriate stream exposure. Collected leaf litter, woody substrates, dead macrophytes, or other organic materials are suitable for determination of fungal biomass and production since these assays are designed to target a broad group of fungi of mainly ascomycetous and basidiomycetous affinities (Gessner and Newell 2002). If the objectives of the study also include estimation of sporulation rate and/or community structure of aquatic hyphomycetes, then headwater streams may be the best choice because of the greater abundance and diversity of these fungi in fast flowing well-aerated streams. Aquatic hyphomycetes can also be found in large rivers (e.g., seventh order; Baldy *et al.* 2002) and specific objectives of the study (e.g., effect of pollution, inorganic nutrients, pesticides, etc. on these fungi) may dictate the choice of site.

B. Sampling Conidia of Aquatic Hyphomycetes from the Water Column

Iqbal and Webster (1973) proposed a straightforward technique to study conidia of aquatic hyphomycetes in transport based on filtering a known volume of water through a membrane filter, staining trapped conidia, and subsequent microscopic examination to count and identify spores. Conidia of aquatic hyphomycetes can often be identified to species due to their characteristic shapes (Gulis *et al.* 2004, Ingold 1975, Marvanová 1997, Webster and Descals 1981). A slightly modified method is described here based on our own experience.

Concentrations of aquatic hyphomycete conidia in stream water can vary over a wide range (100 to 25,000 per liter) (e.g., Gulis and Suberkropp 2005, Suberkropp 1991). The amount of seston carried by the stream also varies considerably over space and time (e.g., Lamberti and Resh 1987). Preliminary trials in a chosen stream may be useful to estimate the volume of water to be filtered in order to trap enough conidia on the filter while preventing interference from too much debris when examining the filter with the microscope. In most streams, 0.2–1.0 liter is a reasonable compromise.

C. Fungi Associated with Plant Litter

Although sporulation rate, fungal biomass and production can be estimated from different types of decaying plant litter, we use here leaf litter in all experimental protocols. Since microbial parameters should be standardized by ash-free dry mass (AFDM) of plant litter, two identical sets of subsamples for biomass and production experiments should be taken (or three sets total if sporulation, biomass, and production are estimated at the same time). Sets of disks can be easily cut from leaves, one set is used to determine AFDM and the other (or other two) for analyses.

D. Sporulation Rate of Aquatic Hyphomycetes

We describe the method to induce sporulation from substrates collected in the field using specially designed laboratory incubation chambers (Figure 15.2) proven to adequately simulate stream conditions with respect to turbulence and aeration necessary to trigger conidia production in many species of aquatic hyphomycetes. The chambers can be ordered from a local glass blower. The alternative is to use Erlenmeyer flasks aerated aseptically through glass tubes or pipettes (Bärlocher 1982, Maharning and Bärlocher 1996) or agitated on a shaker (Baldy *et al.* 1995, Hieber and Gessner 2002). However, the resulting

FIGURE 15.2 Laboratory microcosm used to induce sporulation of aquatic hyphomycetes *(after Suberkropp 1991).*

conidia production estimates from different methods have not been directly compared and may differ somewhat. We strongly recommend filtering conidia suspensions and preparing microscopic slides immediately when laboratory incubations are terminated. However, if a large number of samples are to be processed, suspensions can be fixed with formalin (2% final concentration) in plastic centrifuge tubes and processed later (Hieber and Gessner 2002). It is not known what losses in conidia such a procedure may cause.

E. Fungal Biomass

Determination of fungal biomass by direct microscopic biovolume estimates has been proven to be unsatisfactory, since fungal mycelia grow inside opaque plant tissues and problems with separating mycelium from leaf tissue or clearing leaf tissue result in severe underestimates (Gessner and Newell 2002). ATP and chitin assays have been proposed to quantify fungal biomass, but, because of complexity of analysis, lack of selectivity, and/or other pitfalls, they will not be considered here. The method adapted for this chapter is based on determination of the specific biochemical marker, *ergosterol*, which is the major membrane sterol in higher fungi (Gessner and Newell 2002). Ergosterol is thought to be a measure of living biomass since it is prone to fast degradation upon cell death and membrane disruption.

Liquid-to-liquid extraction of ergosterol from plant litter samples (Newell *et al.* 1988 as modified by Suberkropp and Weyers 1996) is a commonly used technique. Caution should be exercised to protect samples from sunlight as UV degradation of ergosterol will occur. Ergosterol is first extracted and saponified by refluxing plant material in alcoholic KOH. The lipid fraction is partitioned into a non-polar solvent and evaporated to dryness. Ergosterol is redissolved in methanol, filtered, and further separated and quantified by high performance liquid chromatography (HPLC). A second method to extract ergosterol after refluxing and saponification is a solid phase extraction (SPE) using reverse phase extraction columns (Gessner and Schmitt 1996). This method allows more samples to be processed in a given time and has been proven reliable in laboratories with high sample throughput and appropriately trained personnel. However, we do not describe this method here because the exact protocol can vary depending on column manufacturer and it involves acidification of extracts that can cause degradation of ergosterol (Gessner and Newell 2002).

F. Fungal Production

Newell and Fallon (1991) introduced the only method available for determining instantaneous growth rates and biomass production for litter decomposing fungi. This method has been modified for use with stream fungi (Gessner and Chauvet 1997, Suberkropp and Gessner 2005, Suberkropp and Weyers 1996) and involves determining the rate of incorporation of radiolabelled acetate into ergosterol. Fungal growth rates are proportional to rates of acetate incorporation into ergosterol and can be calculated using empirical or theoretical conversion factors (Gessner and Newell 2002). Fungal production is then calculated by multiplying growth rates by biomass (determined from ergosterol concentrations).

Leaf disks may be taken from leaves contained in litter bags to determine production during decomposition (Weyers and Suberkropp 1996) or from naturally occurring leaves to determine fungal production for an entire stream reach (Suberkropp 1997). Leaf disks

are incubated in stream water to which radiolabelled acetate is added. Samples are aerated during incubation for 2–5 h depending on the level of activity. Leaf disks are placed in methanol and later ergosterol is extracted and separated with HPLC. The ergosterol fraction is collected and its radioactivity determined with a scintillation counter. All steps are carried out using proper procedures and precautions for handling radioactive samples and waste.

III. SPECIFIC METHODS

A. Basic Method 1: Conidia of Aquatic Hyphomycetes in Water Column

Field Protocol

1. Take a water sample of 0.2–1.0 liter directly with a graduated cylinder if possible or subsample a larger grab sample. Care should be taken to not disturb benthic sediments. It is preferable to sample riffles since there are indications that shallow calm pools can serve as sinks for conidia. Filter the water sample immediately through a mixed cellulose ester membrane filter (47 mm diam., 5-8 μm pore size) using a hand pump and applying a gentle vacuum (less than 20 cm Hg) to avoid distortion of aquatic hyphomycete conidia.
2. Release the vacuum and cover the filter with a solution of trypan blue (or cotton blue) in lactic acid with a dropper. Pull the excess stain through the filter with vacuum and transfer the filter into a Petri dish with a tight-fitting lid, conidia side up. Filters should stay moist and can be stored for several months.
3. Rinse the cylinder and filter holder with stream water between samples.

Laboratory Protocol

1. Cut the filter in half, mount each half on a microscopic slide with 2–3 drops of the trypan blue (or cotton blue) stain, and add a 22×40 mm coverslip. Avoid lateral movement of the coverslip since this may distort conidia.
2. Scan filters with a compound microscope at 150–200× magnification to count and identify conidia of aquatic hyphomycetes. Record the number of microscopic fields counted per filter.
3. Use Table 15.1 to enter data. We recommend scanning at least 150 microscopic fields (area *ca.* 1 mm^2 each) per filter or until at least 200 conidia are counted.

Data Analysis

1. Aquatic hyphomycete conidia concentration (C) in water (no./L) can be calculated as:

$$C = \frac{n \cdot A_e}{f \cdot A_{mf} \cdot V} \tag{15.1}$$

TABLE 15.1 Sample Data Sheet for Enumeration of Aquatic Hyphomycete Conidia on Filters (some data are filled in as guidance)

Stream_____ Date_____ Filter ID_____

Volume filtered (L)0.5

Effective filtered area (mm^2)__1018 Microscopic field area (mm^2)__0.92

Aquatic hyphomycete	Conidia counted		
	Half A	Half B	A+B
Alatospora acuminata	15	13	28
Anguillospora longissima	25	19	44
. . . . . etc.	. . . . .	. . . . .	. . . . .
Total conidia counted	105	98	203
Number of species	16	15	19
Fields counted	75	75	150

where n is the number of conidia counted; A_e, effective filtered area (determined from the inside diameter of the filter holder in mm^2); f, number of fields counted; A_{mf}, microscopic field area (mm^2); V, volume of water filtered (L).

2. To analyze community structure of aquatic hyphomycetes or to make comparisons between streams, traditional metrics such as diversity, evenness, and similarity can be calculated based on relative abundances of conidia of individual species. To account for differences in sample sizes when comparing species richness of aquatic hyphomycetes, rarefaction as commonly used for stream invertebrates (i.e., based on number of individuals collected) can be used. The alternative is to standardize on the volume of water from which conidia were counted rather than on the number of individuals (conidia) counted (see Bärlocher and Graça 2002, Gulis and Suberkropp 2004).

B. Basic Method 2: Sporulation Rate of Aquatic Hyphomycetes

Field Protocol

1. Take a water sample (acid-washed plastic bottle) sufficient to have 40 mL for each laboratory microcosm used to induce sporulation.
2. Collect submerged decaying leaves, rinse them in the stream, and either transport to the laboratory in stream water (or Ziplock bags) in a cooler or process in the field as follows.
3. For each sample, cut 10 leaf disks with an 11.2 mm diameter cork borer avoiding main veins. Place disks in a wide-mouth jar filled with stream water and transport back to the laboratory in a cooler.

Laboratory Protocol

1. Filter stream water (Whatman GF/F) and use 40 mL to fill each incubation chamber (Figure 15.2).
2. Place a set of 10 disks in each sporulation chamber, adjust air flow to 80–100 mL/min, and incubate at 15°C (or prevailing stream temperature) for

24±2h. Rather strict standardization of incubation time is necessary since sporulation rate through time may be nonlinear.
3. Stop aeration and record incubation time (±5 min). Slowly drain the conidia suspension into a 100-mL beaker rinsing the inner walls of the chamber with the suspension (5-mL pipettor) to remove attached conidia.
4. Add 100 µL of Triton X-100 solution and a stirring bar to each beaker and stir gently for several minutes (150–200 rpm) to achieve uniform distribution of conidia.
5. Take a 2–20 mL aliquot (or make dilutions if necessary), transfer onto 25 mm diameter, 5–8 µm pore size membrane filter and apply gentle vacuum. Rinse the walls of the funnel with distilled water so all conidia are collected and prepare the filter as described in Basic Method 1. Mount the entire filter on a microscopic slide applying a drop of stain on top of the filter and add a 22 × 22 mm coverslip.
6. Transfer leaf disks from each incubation chamber into preashed and preweighed small crucible or aluminum weighing boat and dry to a constant weight at 100°C (e.g., 2 d). Let cool in a desiccator for 10 min and weigh to the nearest 0.1 mg. Combust at 500°C for at least 4 h or overnight, reweigh and calculate AFDM by difference (see also Chapter 17).
7. All prepared filters should be checked under the microscope (*ca.* 15 min is needed to stain conidia intense blue) to ensure that the appropriate density of conidia is achieved. Conidia concentration and the amount of debris interfering with counting and identification can vary greatly. In some cases, it will be necessary to prepare another filter from the same sample and adjust the volume filtered or make a dilution.
8. At least 10 microscopic fields (*ca.* 1 mm^2 each) and at least 200 conidia should be counted and identified at 150–200× magnification. Use a modification of Table 15.1 (not provided) to enter results. A substantially lower number of conidia is acceptable for early (less than a week) and late stages of leaf litter decomposition since sporulation rates are typically very low at these times.

Data Analysis

1. Sporulation rate S (conidia mg^{-1} AFDM d^{-1}) is calculated as:

$$S = \frac{n \cdot A_e \cdot V_c}{f \cdot A_{mf} \cdot V_a \cdot m \cdot t} \tag{15.2}$$

where n is the number of conidia counted; A_e, effective filtered area (mm^2); V_c, volume of fluid in chamber (mL); f, number of fields counted; A_{mf}, microscopic field area (mm^2); V_a, aliquot of conidia suspension filtered (mL); m, AFDM of 10 disks used to induce sporulation (mg); t, incubation time (d).
2. Conidia production can also be expressed in terms of dry mass or fungal carbon (Baldy *et al.* 2002, Gulis and Suberkropp 2003b) using published values on dry masses of conidia of dominant aquatic hyphomycetes (Chauvet and Suberkropp 1998) or biovolume estimates (Bärlocher and Schweizer 1983).

C. Advanced Method 1: Fungal Biomass

Preparation Protocol

1. Prepare ergosterol standards in methanol (e.g., in the range of 2-25 μg/mL) and store at −20°C.
2. Prepare alcoholic KOH solution. Dissolve 4.8 g KOH in 6 mL distilled water. When KOH has dissolved, add 114 mL methanol, which is sufficient for 20 samples.

Field Protocol

1. Collect leaf litter as described in Basic Method 2.
2. For each sample cut 2 sets of 5 leaf disks each. Preserve one set in 5 mL of methanol in a 20-mL glass scintillation vial. Transport to the laboratory in a cooler and then store in a freezer (−20°C) for up to several months. Use the corresponding set of leaf disks for AFDM determination.

Laboratory Protocol

1. Determine AFDM of the corresponding set of disks as described in Basic Method 2.
2. *Ergosterol extraction*. Transfer leaf disks and 5 mL of methanol in which they were stored to round bottom flasks. Use 5-mL aliquots of methanol to wash vials. Make final volume to 25 mL. Add 5 mL of alcoholic KOH and a boiling chip.
3. Attach flasks to reflux condensers, turn on water to condensers, and place flasks in the water bath at 70°C. Reflux for 30 min.
4. Remove flasks from the water bath and let cool. Remove and dispose of leaf disks and boiling chips using forceps. Transfer extract from each flask to a 65-mL screw-top tube and add 10 mL of deionized water.
5. Use 10 mL of pentane to wash each round bottom flask and add to methanolic extract in screw-top tube. Screw cap on tightly. Mix thoroughly by inverting tubes at least 30 times (or in a Rotamix for 3 min at 20 rpm). Remove pentane (upper phase) with a Pasteur pipette into a 15-mL conical centrifuge tube. In a fume hood, evaporate pentane in tube heater at 30°C under a stream of N_2 delivered through syringe needles.
6. Repeat the partitioning into pentane step twice by adding 5 mL of pentane to methanol phase in the screw-cap tube, mixing, removing top phase to the same centrifuge tube, and evaporating as above. Discard methanol phase.
7. Evaporate pentane to dryness and redissolve residue in 1 or 2 mL of methanol (use a precise syringe, as accuracy is critical). Sonicate in bath sonicator (twice for 5 min or until no visible residue on tube walls).
8. Filter ergosterol extract through 0.2-μm syringe filter (PTFE) into HPLC vial. Extracts can be stored at −20°C for several months until analyzed by HPLC.
9. *HPLC purification and quantification of ergosterol*. Sonicate extracts to degas (5 min) after removal from a freezer. Set a UV detector to 282 nm. Use methanol as a mobile phase and set flow rate to 1.0–1.5 mL/min. Inject 20 μL of each ergosterol standard followed by sample extracts. Retention time of ergosterol varies depending on flow rate, temperature, and column properties and usually is 4–10 min. Use peak area of ergosterol standards to perform regression and calculate ergosterol concentrations of samples.

Data Analysis

1. Fungal biomass B_f (mg/g AFDM) associated with plant litter can be calculated as:

$$B_f = \frac{C_e \cdot V_e}{m \cdot 5.5} \tag{15.3}$$

where C_e is ergosterol concentration of sample extract from HPLC (μg/mL); V_e, volume of extract (i.e., 1 or 2 mL); m, AFDM of corresponding set of 5 leaf disks (mg); 5.5 is ergosterol to biomass conversion factor (5.5 mg ergosterol g^{-1} fungal dry mass) (Gessner and Chauvet 1993).

2. If community structure of aquatic hyphomycetes is known (Basic Method 2), species-specific conversion factors may be applied to calculate fungal biomass (Baldy *et al.* 2002, Gessner and Chauvet 1993). It is not clear, however, whether conidial production of individual species from leaf litter correlates with their contribution to mycelial biomass within substrates.

D. Advanced Method 2: Fungal Production

Preparation Protocol

1. Prepare [1-^{14}C]acetate plus nonradioactive sodium acetate stock solution. The stock solution is made so that 50 μL contains 1 MBq of [^{14}C] acetate and the total acetate concentration is 0.4 M.
2. Filter stream water through a membrane filter (0.45 μm pore size) and distribute 3.95 mL to each incubation tube equipped with an aeration tube.

Field protocol

1. Collect leaf litter as described in Basic Method 2.
2. For each sample, cut two sets of 5 leaf disks each. Place one set in an incubation tube containing 3.95 mL of filtered stream water and use the corresponding set for AFDM determination.
3. One extra tube containing leaf disks will be used as the killed control to determine background levels of radioactivity in the ergosterol fraction. After leaf disks have been placed in this tube, add formalin to a final concentration of 2%.
4. Place tubes in a rack in the stream, connect aeration tubes to a battery operated pump and aerate each tube with 30–40 mL air/min. If handling radioactivity in the field is not possible, then tubes and disks should be placed in a cooler close to stream temperature and transported back to the laboratory where the tubes can be placed in a water bath adjusted to stream temperature in a fume hood and aerated.
5. Allow the disks to equilibrate for 10–20 min and add 50 μL of [^{14}C]acetate solution to each tube at timed intervals and incubate for a precise time (120–300 min).
6. At timed intervals, remove the tubes from the stream in the same order as addition of acetate occurred and place in an ice bath to slow additional uptake of acetate.

7. Filter the contents of each tube through glass fiber filters (25 mm diam). Rinse with filtered stream water and place filters and leaf disks in 5 mL methanol. Transport to the laboratory on ice and store at −20°C until ergosterol is extracted.

Laboratory Protocol

1. Extract and measure ergosterol as described in Advanced Method 1 with modifications described below. Make certain to follow proper protocol for handling radioactive samples and waste.
2. Keep the final volume of the ergosterol extract as small as possible (0.5–1.0 mL) and inject larger volumes (100–250 µL) into the HPLC than used for ergosterol determination (Advanced Method 1). Multiple injections of the same sample are also recommended. All these precautions increase the sensitivity of the assay since the radioactivity of the ergosterol fraction is typically very low.
3. Collect the ergosterol peak eluting from the HPLC in a scintillation vial.
4. Add 10 mL of scintillation fluid to the combined ergosterol fractions from each sample.
5. Determine radioactivity of ergosterol with a scintillation counter and correct for quenching.

Data Analysis

1. Calculate ergosterol concentrations from peak areas and standard curve as in Advanced Method 1.
2. Calculate the fungal growth rate (k) (mg mg^{-1} d^{-1}) as:

$$k = \frac{(R_s - R_c) \cdot 19300}{a_s \cdot F \cdot t \cdot B_{fs}} \tag{15.4}$$

where R_s is the radioactivity (Bq) in the sample; R_c, radioactivity (Bq) in the control; a_s, specific activity (Bq/mmol) of the acetate; F, fraction of the sample that is injected into the HPLC; t, incubation time (d); B_{fs}, fungal biomass in the sample (mg); 19300 is an empirically derived conversion factor (mg fungal biomass mmol^{-1} acetate incorporated) (Suberkropp and Weyers 1996). See Gessner and Newell (2002) for other conversion factors.

3. Fungal production (P_f) (mg g^{-1} leaf AFDM d^{-1}) is calculated as:

$$P_f = k \cdot B_f \tag{15.5}$$

where k is the growth rate (mg mg^{-1} d^{-1}) and B_f is the fungal biomass (mg g^{-1} leaf AFDM).

IV. QUESTIONS

1. What stream characteristics affect concentrations of aquatic hyphomycete conidia in water?
2. Why does conidia concentration in stream water usually exhibit a clear seasonal pattern? Would you expect to find seasonal pattern in tropical, arctic, or desert streams? Would you expect to find diel fluctuations in conidia concentration?
3. Are species identified from water samples similar to those recorded from laboratory sporulation experiments from the same stream? Why or why not?
4. Why is it important to use filtered stream water for laboratory incubations to induce sporulation of aquatic hyphomycetes?
5. Did you notice significant differences in sporulation rates of aquatic hyphomycetes from different types of leaf litter (substrates)? Why or why not?
6. How does nutrient concentration in water, siltation, or algal development affect fungal activity on submerged plant litter?
7. What interactions may exist between aquatic fungi and stream invertebrates?
8. What implications do riparian management practices or land use in the watershed (logging, agriculture, urban development, etc.) have on fungal activity in streams?

V. MATERIALS AND SUPPLIES

Letters in parenthesis indicate in which Basic (B1 or B2) or Advanced (A1 or A2) Method the item is used.

Field Materials

[1-^{14}C]acetate, sodium salt and noradioactive sodium acetate (A2)
Acid-washed plastic bottle (B2)
Air flow meters (A2)
Battery-operated air pump (A2)
Cork borer (e.g., 11.2 mm diam.) (B2, A1, A2)
Filter holders to accommodate 47-mm diam. filters (B1) and 25-mm diam. filters (A2)
Formalin (A2)
Glass fiber filters (25-mm diam.; e.g., Whatman 934-AH) (A2)
Glass scintillation vials (20 mL) (A1, A2)
Graduated cylinder (500 mL) (B1)
Hand pump with manometer (B1)
Ice (A2)
Membrane filters (47-mm diam., 5–8 μm pore size; e.g., Millipore, white SMWP) (B1)
Petri dishes with tight-fitting lids (50-mm diam.; e.g., BD Falcon) (B1)
Receiving flasks (B1, A2)
Stain dropper (B1)
Sterile filter apparatus and membrane filters (0.45 μm pore size) (A2)
Forceps (B1, B2, A1, A2)
Trypan blue (Sigma) in lactic acid (0.05%) or cotton blue (Sigma) in lactic acid (0.1%) (B1, B2)

Tubes (40 mL) fitted with 2-holed rubber stoppers, glass and rubber tubing for aeration, and a tube rack (A2)
Wide-mouth jars (50 mL) (B2)
Ziplock bags (optional) (B2, A1)

Laboratory Materials

Beakers (100 mL) (B2)
Conical glass centrifuge tubes with screw caps (15 mL) (A1, A2)
Ergosterol (purity ≥98%; e.g., Alfa Aesar)
Filter holder to accomodate 25-mm diam. filters (B2)
Glass fiber filters (Whatman GF/F) (B2)
Glass scintillation vials (20 mL) (A2)
Glass syringes (0.5, 1, 5 mL) (A1, A2)
HPLC vials with teflon-lined caps (2 mL) (A1, A2)
KOH (A1, A2)
Membrane filters (25-mm diam, 5–8 μm pore size; e.g., Millipore, white SMWP) (B2)
Methanol (HPLC grade) (A1, A2)
Microscope slides and coverslips (22 × 22 mm and 22 × 40 mm) (B1, B2)
Receiving flask (1 L) (B2)
Round bottom flasks (100 mL) (A1, A2)
Reflux condensers (A1, A2)
Pentane (HPLC grade) (A1, A2)
Scintillation fluid (e.g., Ecolume) (A2)
Screw top tubes with teflon lined caps (65 mL) (A1, A2)
Small crucibles or aluminum weighing boats (B2, A1, A2)
Syringe filters (13-mm diam, 0.2 μm pore size, PTFE) (A1, A2)
Teflon boiling chips (A1, A2)
Forceps (B1, B2, A1, A2)
Triton X-100 (Sigma) solution (0.5%) (B2)
Trypan blue in lactic acid (0.05%) or cotton blue in lactic acid (0.1%) (B1, B2)

Laboratory Equipment

Analytical balance (B2, A1, A2)
Adjustable pipettors (200 μL, 1 mL, and 5 mL) (B2, A1, A2)
Compound light microscope (B1, B2)
Drying oven (B2, A1, A2)
Dry tube heater (A1, A2)
HPLC system [pump for isocratic operation, autosampler, or 20 μL injection loop (A1) or 100 μL injection loop (A2), UV detector, integrator, or computer with software package (A1, A2) and automatic fraction collector (A2)]
HPLC column (reverse phase C_{18}; e.g., 25 × 4.6 mm, Whatman Partisphere) (A1, A2)
Magnetic stirrer and stirring bars (B2)
Muffle furnace (B2, A1, A2)
Rotating tube mixer (Rotamix) (A1, A2)
Scintillation counter (A2)
Sonication bath (A1, A2)
Tank of nitrogen gas and manifold with syringe needles (A1, A2)
Vacuum pump/connection (B2)
Water bath (70°C) (A1, A2)

VI. REFERENCES

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