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HAL Id: hal-00870750

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To link to this article : DOI:10.4319/lo.1997.42.3.0496
URL : <http://dx.doi.org/10.4319/lo.1997.42.3.0496>

To cite this version : Gessner, Mark O. and Chauvet, Eric. *Growth and production of aquatic hyphomycetes in decomposing leaf litter*. (1997) *Limnology and Oceanography*, vol. 42 (n° 3). pp. 496-505. ISSN 0024-3590

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Growth and production of aquatic hyphomycetes in decomposing leaf litter

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Abstract

The acetate-to-ergosterol technique was used to estimate fungal productivity of three species of aquatic hyphomycetes growing in decomposing ash leaves in stream microcosms. Following a lag of 20–88 min, incorporation of acetate into ergosterol was linear for at least 10 h. Substrate saturation was reached in the mM range, and there was no indication of isotope dilution. For one species, *Articulospora tetracladia*, a conversion factor of 5.5 mg mycelial dry mass produced per μmol acetate incorporated was determined. This was similar to the theoretical conversion factor ($6.6 \text{ mg } \mu\text{mol}^{-1}$) deduced from pathways of ergosterol synthesis in fungi. Thus, the acetate-to-ergosterol assay appears to be suitable for estimating the productivity of aquatic hyphomycetes growing in leaf litter in streams. Estimated growth rates of *A. tetracladia* in microcosms changed markedly over time, with the maximum being as high as 0.72 d^{-1} at an early growth stage. After 23 d when 58% of the initial leaf mass was degraded, the fungus had produced 89 mg biomass per g of initial leaf mass. Almost half of this production was allocated to conidia. Assuming an average growth efficiency of 0.35, this would be equivalent to a fungal assimilation of 25% of initial leaf mass and account for 44% of the observed leaf mass loss. In an experiment with leaf litter colonized by fungi in a stream, acetate incorporation was linear for 6 h, but the estimated growth rate was only 0.017 d^{-1} .

A critical step in ecosystem analysis is the assessment of biomass production of the major species or functional groups. In addition, as Benke (1993, p. 15) stated in his review on animal secondary productivity in running waters: "Production is the most comprehensive representation of 'success' for a population." Measuring productivity of single species and species assemblages is hence critically important in ecology, regardless of whether questions are addressed from an organismic perspective or at the ecosystem level. Not surprisingly therefore, the development and use of methods to measure in situ phytoplankton and bacterial productivity (Hurst et al. 1996) have significantly influenced current views of the structure and functioning of both pelagic and benthic aquatic systems (Hobbie 1994; Schwoerbel 1994).

In streams, one of the major energy sources for overall metabolism is derived from riparian trees and shrubs, with leaf litter being a particularly important fraction (Cummins

1988; Schwoerbel 1994). This allochthonous resource is rapidly colonized and exploited by a consortium of fungi, bacteria, and detritivorous animals known as shredders (Webster and Benfield 1986; Boulton and Boon 1991). Although shredders may be important in the breakdown of leaf litter in streams (Maltby 1992), recent evidence suggests that a major fraction of this material is diverted to secondary production of saprotrophic fungi (Baldy et al. 1995; Suberkropp 1995) and especially of a group commonly referred to as aquatic hyphomycetes (Bärlocher 1992; Suberkropp 1992a). Fungal biomass associated with decomposing leaf litter can in fact exceed 15% of total detrital mass (Gessner and Chauvet 1994; Suberkropp 1995) and accounts for more than 90% of the total microbial (bacterial plus fungal) biomass (Findlay and Arsuffi 1989; Baldy et al. 1995). In addition, aquatic hyphomycetes allocate a substantial portion of assimilated organic matter to nonsexual propagules (up to seven conidia per μg detrital dry mass per day; Suberkropp 1991; Gessner and Chauvet 1994; Bärlocher et al. 1995). Because of this allocation to, and subsequent release of, conidia, losses of mycelial mass due to fragmentation (Suberkropp and Klug 1980) and selective feeding of detritivores on fungal hyphae (Suberkropp 1992b), the overall productivity of aquatic hyphomycetes cannot be adequately assessed by simply measuring differences in fungal biomass.

Until recently, a satisfactory evaluation of fungal productivity was hampered by a lack of appropriate methodology. Newell and Fallon (1991) therefore devised a technique to measure virtually instantaneous growth rates and production of fungi colonizing standing-dead emergent macrophytes in marine and freshwater wetlands. The rationale underlying their method is similar to that adopted for estimating bac-

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Acknowledgments

We thank S. Y. Newell and K. Suberkropp for discussion, and M. Escautier and A.-M. Jean-Louis for technical assistance during portions of this study.

Financial support for this research was provided by the French Ministry of the Environment (EGPN; grant 92221), the Regional Council of Midi-Pyrénées (CCRD; grants RECH9300082 and RECH9307903), and the German Federal Ministry of Research and Technology (BMFT; grant 0339077E). Travel grants were made available through the French–German cooperation program PROCOPE (references 312/pro bmbw gg and 94074).

terial productivity via measurements of [³H]thymidine and [³H]leucine incorporation into DNA and protein, respectively (Hurst et al. 1996), in that it is based on the incorporation of a radiolabeled precursor into a major cell constituent of the target organism (Newell 1994). Specifically, [¹⁴C]acetate is incorporated into ergosterol as the major sterol of higher fungi (Newell 1992). One of the advantages of the acetate-to-ergosterol technique is that the labeled endproduct, ergosterol, is, for all practical purposes, restricted to the target organism (Newell 1992), so that specificity can be achieved by isolating a chemically defined molecule.

The primary objective of this study was to adapt the acetate-to-ergosterol method to the decomposing leaf litter system in streams, whose microbial community is dominated by aquatic hyphomycetes. Before considering application to field conditions, we decided to test the method in a simple model system (microcosm) mimicking the conditions of leaf litter decomposition in streams. Our specific intentions were to check whether basic assumptions underlying the acetate-to-ergosterol method are met in our study system, to provide estimates of instantaneous growth rates and production of aquatic hyphomycetes associated with decomposing leaf litter, to construct an organic matter budget for a saprotrophic fungus growing in a stream microcosm, and, by comparing the dynamics of mycelial growth and sporulation, to gain insight into the life history pattern of aquatic hyphomycetes.

Material and methods

Single spore isolates of three aquatic hyphomycetes, *Articulospora tetracladia* Ingold (isolate CERR 29.73), *Lunulospora curvula* Ingold (CERR 80.146), and *Tetracladium marchalianum* de Wildeman (CERR 78.152), were obtained from foam or submerged leaf litter collected in streams of southwestern France. Cultures were maintained on 2% malt agar.

To mimic natural conditions of fungal development in leaf litter, fungi were grown in a laboratory model system consisting of a glass chamber connected to an aeration tube, which provides a constant flow of cotton-filtered air (about 100 ml min⁻¹), and an outlet allowing aseptic drainage (Suberkropp 1991). Chambers were stocked with 20 air-dried, preweighed leaf discs (10 mm diameter) previously cut from freshly shed, yellow-senescent ash leaves (*Fraxinus excelsior* L.). Forty milliliters of deionized water were added and the chambers autoclaved for 15 min at 121°C. After cooling, the liquid was drained off and the chambers refilled with a mineral salt solution consisting of 0.1 g CaCl₂·2H₂O, 0.01 g MgSO₄·7H₂O, 0.01 g KNO₃, 0.55 mg K₂HPO₄, and 0.5 g MOPS (3-morpholinopropanesulfonic acid) buffer in 1 liter of water (pH adjusted to 7.0). Conidial suspensions for inoculation of the chambers were obtained by submerging pieces of agar cut from the growing edge of a fungal colony in culture medium (Webster and Descals 1981). The conidial densities were determined by filtering known volumes of the suspensions over 5-μm membrane filters (Suberkropp 1991), staining the trapped conidia with 0.01% trypan blue in lactic acid and counting at a magnification of 200. Chambers were then inoculated with aliquots of the suspensions correspond-

ing to 4,000–6,000 conidia. The system was aerated for 5 min to ensure a uniform distribution of conidia and then allowed to settle for 1 h before aeration was started again. After 24 h and at subsequent intervals of 2–3 d, the liquid was changed, two replicate aliquots filtered over membrane filters (Suberkropp 1991), and the trapped conidia counted as described above. Total conidial mass was calculated from conidial numbers and the individual mass of conidia that had been previously determined by weighing a large number of conidia filtered on a tared membrane filter (Suberkropp 1991; Suberkropp and Chauvet unpubl. data).

Instantaneous growth rates (μ) of fungi associated with leaf discs were determined by the acetate-to-ergosterol assay devised by Newell and Fallon (1991) (Newell 1993). For each experiment, leaf discs of several chambers were first pooled and then randomly reappportioned to 20-ml polyethylene vials with each vial receiving 10 discs. Discs were incubated in 5 ml of an acetate solution made up in either culture medium or stream water with final added acetate concentrations ranging from 0.16 to 10 mM. The incorporation of radiolabel into ergosterol was started by adding 100–300 μl (equivalent to 1–2 MBq) of an aqueous sodium [1-¹⁴C]acetate solution (Amersham, CFA.13). The radiolabeled acetate was added immediately after the cold acetate in all but three experiments, in which the delay was 0.5–1 h. Control vials received 300 μl of 37% formaldehyde 30–60 min prior to the addition of radioactivity. Incubations were carried out for 3 h or, in time-course experiments, for 1–10 h at 15°C in the dark with gentle shaking (75 rpm, 26-mm amplitude). After stopping the incorporation of radiolabel by adding 300 μl formaldehyde, the leaf discs were immediately collected on a 5-μm membrane filter, rinsed three times with 2 ml of deionized water, frozen at -18°C, later lyophilized, weighed to the nearest 0.1 mg, and subjected to ergosterol extraction.

Ergosterol and other lipids were extracted from leaf discs by 30 min of refluxing in alcoholic base (Gessner et al. 1991). Lipids were partitioned into petrol ether, evaporated to dryness under a stream of nitrogen, and redissolved in 250 μl of dichloromethane:isopropanol (99:1, vol:vol). Separation of ergosterol from matrix lipids was achieved by means of HPLC with UV detection at 282 nm. The chromatographic system consisted of a Kontron pump (model 422), a model 360 autoinjector equipped with a 10-μl sample loop, and a variable wavelength detector (model 432). The column was a 25-cm × 4.6-mm RP18 Lichrospher (endcapped, 5-μm particle size). Column temperature was maintained at 38°C. The system was run isocratically with HPLC-grade methanol at a flow rate of 1.5 ml min⁻¹. Ergosterol eluted between 7.7 and 8.6 min. It was quantified by measuring peak height, and peak identity was checked on the basis of retention times of pure ergosterol purchased from Fluka (purity >98%). The ergosterol fractions of three injections from the same sample were pooled in a scintillation vial, 10 ml of fluor (Beckmann ReadySolv) was added, and radioactivity measured on a Packard Tri-Carb 1900 TR liquid scintillation analyzer with automatic quench correction. Ergosterol concentrations were converted to fungal biomass of *A. tetracladia* with a strain-specific conversion factor de-

Table 1. Selected physical and chemical characteristics of stream water and of culture medium in microcosms. Values for the stream refer to the end of the incubation period (day 11) except for temperature where the two values correspond to day 0 and 11, respectively. Values for microcosms refer to freshly prepared culture medium.

Parameter	Stream	Microcosms
Temperature (°C)	4–5	15
pH	7.4	7.0
Conductivity ($\mu\text{S cm}^{-1}$, 25°C)	26.2	
Alkalinity (mmol liter^{-1})	0.204	
[NO ₃ -N] (mg liter^{-1})	0.20	1.39
[PO ₄ -P] ($\mu\text{g liter}^{-1}$)	19	98

terminated previously (5.0 mg g^{-1} mycelial dry mass; Gessner and Chauvet 1993).

A field test of the acetate-to-ergosterol method was performed with leaf material naturally colonized in a softwater mountain stream. Physical and chemical characteristics of the stream water during the colonization period (Table 1) were similar to those in previous years (Gessner and Chauvet 1994 and references therein). On 27 November 1992, a total of 120 leaf discs (*F. excelsior*) were enclosed in 12 separate compartments of nylon mesh bags (1.5-mm mesh) and submerged in the stream. They were retrieved after 11 d and placed in polypropylene boxes containing stream water. In the laboratory, leaf discs were pooled, carefully rinsed with stream water, and divided into 12 batches of 10 discs each. Six batches were used to measure instantaneous mycelial growth rates with the acetate-to-ergosterol method. Three of these were incubated in culture medium as described above, whereas incubation of the other three batches was carried out in filtered ($0.2 \mu\text{m}$) stream water. Incubations were carried out at 15°C. The remaining six batches were aerated in growing chambers, three of which contained culture medium and the other three filtered stream water. The conidia released from leaves within 45 h in the chambers were trapped on membrane filters, stained, identified, and counted. Dry mass and ergosterol concentrations of leaves from the latter 6 batches were determined after lyophilization.

Instantaneous growth rates, μ , were calculated according to $\mu = \ln(1 + P/B)$. This relationship is derived from the equation $P = B_{t+\Delta t} - B_t$, where P is the production in a given time period, t , and B_t the biomass at time t , and the exponential growth model $B_t = B_0 \cdot \exp(\mu \cdot t)$, where B_0 is the initial biomass.

Data were analyzed by linear and nonlinear regression analysis, Student's t -test, analysis of variance, and analysis of covariance. All calculations were done with SYSTAT (Wilkinson 1990). The significance level was set at $P = 0.05$ except when noted otherwise. To determine whether isotope dilution occurred in saturation experiments, it was tested (t -test) whether the intercept, b , of the linear function $y = ax + b$, where y is the concentration of added acetate, a is the slope, and x is the reciprocal of incorporated radioactivity per unit detrital mass or ergosterol, deviated significantly from zero.

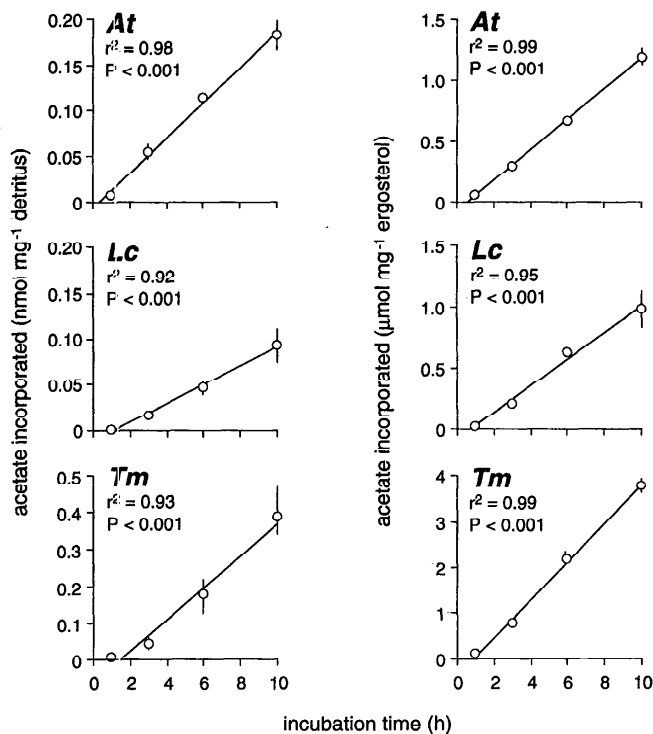


Fig. 1. Time-course of acetate incorporation by three species of aquatic hyphomycetes growing in ash leaf litter in stream microcosms. Error bars indicate ranges of two to three replicate measurements. Detritus refers to the sum of leaf mass and fungal mass. At, *A. tetracladia*; Lc, *L. curvula*; Tm, *T. marchalianum*.

Results

Linearity of incorporation as a function of elapsed time—When aquatic hyphomycetes were grown in ash leaves in microcosms, incorporation of acetate into ergosterol followed linear kinetics for 10 h irrespective of whether results are expressed on the basis of detrital dry mass (Fig. 1, left panel) or amount of ergosterol (Fig. 1, right panel). Linear regressions were highly significant for all fungal species examined ($P < 0.001$). Correlation coefficients were strongly positive in all cases ($r^2 \geq 0.92$) but consistently higher when results were expressed in terms of ergosterol (Fig. 1, right panel). Incorporation of acetate was notably low initially, resulting in an apparent lag phase of 20–88 min (Fig. 1) and a statistically significant deviation of regression lines from the origin in three of the six cases. As a consequence, estimates of incorporation rates tended to increase with increasing incubation time and thus exhibited significant differences between short and long incubation times (Table 2). Apart from the comparison of *A. tetracladia* and *L. curvula* on an ergosterol basis, the slopes of regression lines varied significantly among species (ANCOVA), with the rate of incorporation being up to four times higher for *T. marchalianum* than for *L. curvula*.

When fungal growth was determined in leaf litter naturally colonized in a mountain stream, incorporation of acetate was also approximately linear for 6 h (Fig. 2). Regressions in-

Table 2. Rates of acetate incorporation into ergosterol as a function of incubation time with radiolabel. *At* = *Articulospora tetradia*; *Lc* = *Lunulospora curvula*; *Tm* = *Tetracladium marchalianum*. Means with identical superscript letters in each column are not significantly different ($P < 0.05$; ANOVA followed by Tukey's test).

Incubation time (h)	Acetate incorporated (pmol mg ⁻¹ detrital mass h ⁻¹)			Acetate incorporated (nmol mg ⁻¹ ergosterol h ⁻¹)		
	<i>At</i>	<i>Lc</i>	<i>Tm</i>	<i>At</i>	<i>Lc</i>	<i>Tm</i>
1	8.6 ^a	1.8 ^a	6.2 ^a	62 ^a	18 ^a	118 ^a
3	18.6 ^b	6.0 ^{a,b}	14.7 ^a	100 ^b	73 ^{a,b}	269 ^b
6	19.0 ^b	7.9 ^{a,b}	29.7 ^b	111 ^b	104 ^b	366 ^c
10	18.3 ^b	9.3 ^b	38.8 ^b	119 ^b	98 ^b	377 ^c

cluding data points over this period were significant ($P < 0.05$, $r^2 = 0.66-0.95$) except for incubations in stream water when expressed in terms of ergosterol ($P = 0.06$, $r^2 = 0.62$). Stream water incubation resulted in higher acetate incorporation rates than incubation in culture medium during that period, but the difference was only significant for incorporation rates expressed on a detrital dry mass basis (ANCOVA, $P < 0.01$). After the first 6 h, incorporation did not follow the same linear kinetics as before, with mixed responses being observed depending on incubation conditions (stream water or culture medium) and mode of expression (detrital dry mass or ergosterol basis). The total amount of acetate incorporated after 10 h per mg ergosterol was even lower than the amount incorporated by 6 h (Fig. 2), though not significantly so. In culture medium, incorporation rates increased sharply between 6 and 10 h, especially when expressed per mg detrital dry mass (Fig. 2).

Sporulation rates of aquatic hyphomycetes in microcosms were significantly higher in culture medium during the 2-d

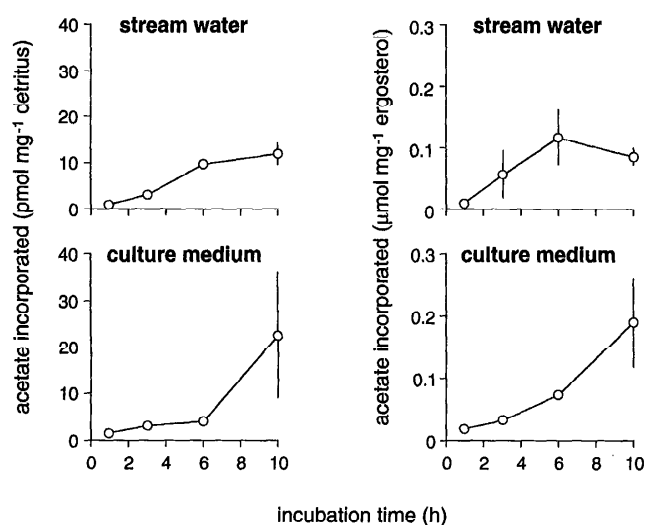


Fig. 2. Time-course of acetate incorporation by a natural fungal assemblage associated with ash leaf litter decomposing in a mountain stream. Error bars indicate ranges of two replicate measurements.

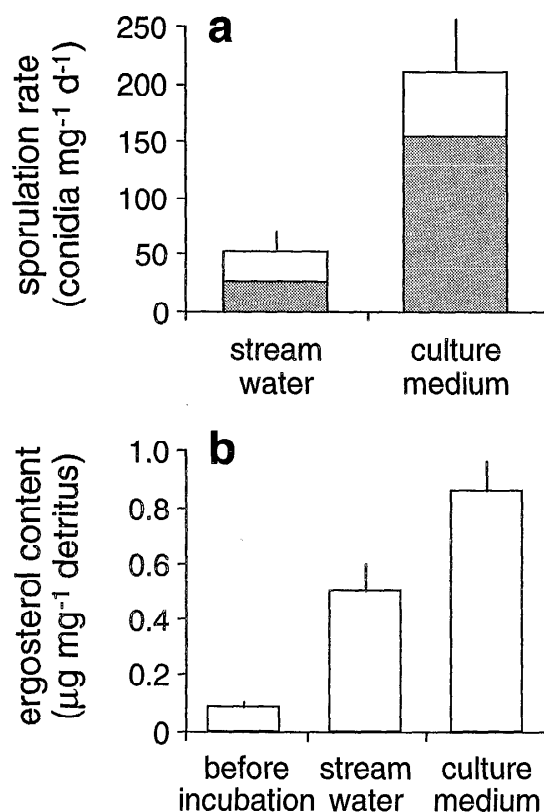


Fig. 3. Sporulation rate of aquatic hyphomycetes associated with ash leaf litter (a) and ergosterol concentration of litter (b) decomposing in a mountain stream. After sampling in the field, leaves were incubated in stream microcosms for 45 h. The shaded bars indicate the proportion of the dominant hyphomycete species, *F. curvula*. Error bars indicate ± 1 SD of three replicate measurements. Differences in treatments were statistically significant in all cases (t -tests).

incubation following sample collection in the field (Fig. 3a). On average, the relative contribution of the dominant species, *Flagellospora curvula* Ingold, to the total conidial output was higher in the culture medium, but this difference was not statistically significant (t -test after angular transformation, $P = 0.11$). Ergosterol concentrations of leaf litter increased 7–11 \times during that 2-d period in microcosms with increases being significantly higher in culture medium than in stream water (Fig. 3b; t -test).

Saturation kinetics and isotope dilution—With acetate concentrations rising from 0.16 to 1 mM, incorporation rates into ergosterol tended to increase when data were expressed on a detrital dry mass basis (Fig. 4, left panel). Incorporation rates leveled off at higher concentrations, or, especially in *T. marchalianum*, increased slightly. The rates determined at high concentrations (5 and 10 mM) nevertheless were not significantly different from those measured at lower concentrations. When expressed on an ergosterol basis, incorporation rates increased in general until 2.5 mM and showed a somewhat erratic pattern at higher concentrations (Fig. 4,

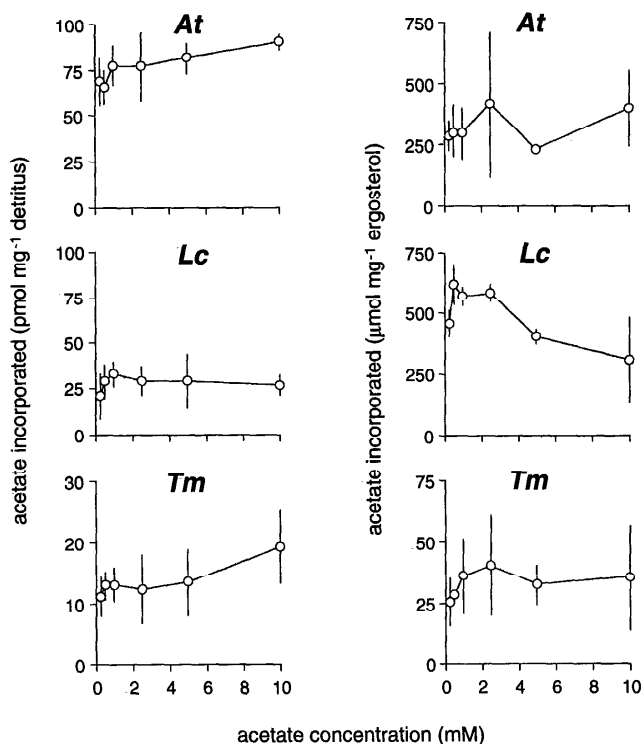


Fig. 4. Acetate incorporation by three species of aquatic hyphomycetes growing in ash leaf litter in stream microcosms as a function of added acetate concentration. Error bars indicate ± 1 SD of three replicate measurements. *At*, *A. tetracladia*; *Lc*, *L. curvula*; *Tm*, *T. marchalianum*.

right panel). Overall, however, there was no indication that acetate incorporation failed to reach saturation levels.

Figure 5 shows isotope dilution plots (Moriarty 1990) of the same data as in Fig. 4. All regression lines were highly significant irrespective of fungal species and mode of expression. The intercepts of regression lines with the abscissas were slightly greater or smaller than zero (range: -0.4 to 0.2 mM) but never significantly different from the origin, indicating that isotope dilution was negligible in all experiments.

Influence of fungal growth stage on acetate incorporation—The ergosterol content of leaves inoculated with *A. tetracladia* was low during the first 5 d of growth in microcosms (Fig. 6a). It rapidly increased thereafter and reached a maximum of 0.42 mg g⁻¹ detrital dry mass after 19 d. At a mycelial ergosterol concentration of 5.0 mg g⁻¹ dry mass (Gessner and Chauvet 1993) this corresponds to a peak mycelial biomass of 8.4% of the detrital mass. The rate of acetate incorporation into ergosterol was highest at day 5 after inoculation (Fig. 6c) concomitant with the lowest amount of mycelial biomass (Fig. 6b) and then decreased sharply to less than 10% of the peak value. Assuming that known pathways of fungal ergosterol biosynthesis (e.g. Weete 1989) apply to *A. tetracladia*, we can convert rates of acetate incorporation to ergosterol production rates. Hence, Fig. 6c also

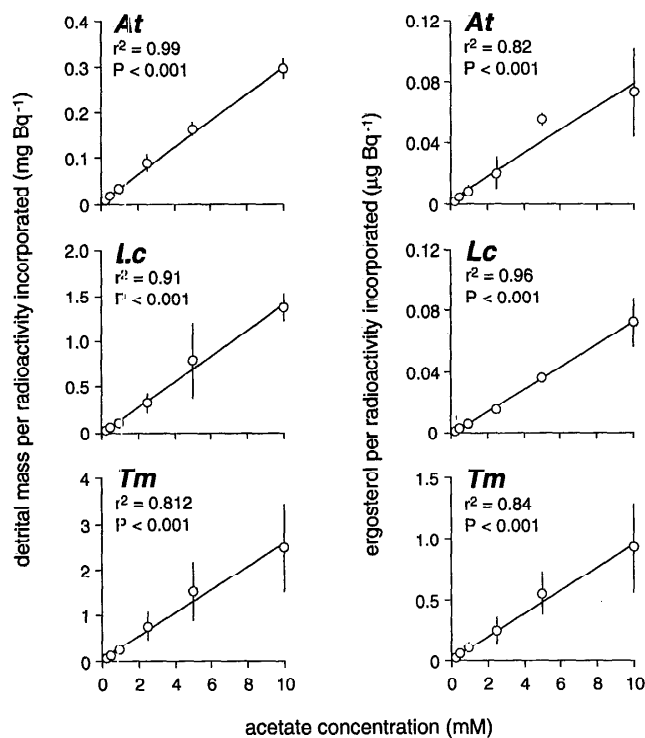


Fig. 5. Isotope dilution plots for three species of aquatic hyphomycetes growing in ash leaf litter in stream microcosms. Error bars indicate ± 1 SD of three replicate measurements. Detrital mass refers to the sum of leaf mass and fungal mass. *At*, *A. tetracladia*; *Lc*, *L. curvula*; *Tm*, *T. marchalianum*.

reflects changes in the specific fungal production rate in leaf litter, i.e. the mg ergosterol (or fungal mass) produced per mg ergosterol (or fungal mass) per unit time, which is also referred to as the *P:B* ratio. Instantaneous fungal growth rates ranged from 0.023 to 0.718 d⁻¹ and, apart from the first two, were thus nearly identical to the corresponding specific production rates (Fig. 6c, e). Owing to the low mycelial biomass after 5 d (Fig. 6b), the rapid growth of *A. tetracladia* at that time (Fig. 6c, e) did not translate into high mycelial production (Fig. 6d). However, daily fungal production increased to a maximum of 9.5 µg fungal mass per mg detrital mass after 9 d (Fig. 6d) when mycelial biomass was only about 24% (concentration, Fig. 6a) and 36% (amount per microcosm, Fig. 6b) of its maximum values. Concomitant with the subsequent diminution in growth rate, fungal production decreased to about one third of the peak value (Fig. 6d), but net accumulation of fungal mass continued until day 19 of the experiment (Fig. 6b). The pattern of change and absolute values of cumulative mycelial production (Fig. 6f) were similar to the dynamics and magnitude of mycelial biomass (Fig. 6b).

Sporulation rates of *A. tetracladia* in microcosms (Fig. 7a) showed broadly similar dynamics as acetate incorporation rates (Fig. 6d) with a relatively rapid increase to a peak value and a subsequent decrease. However, sporulation rates lagged behind acetate incorporation rates by several days. The cumulative conidial output (Fig. 7b), which reports

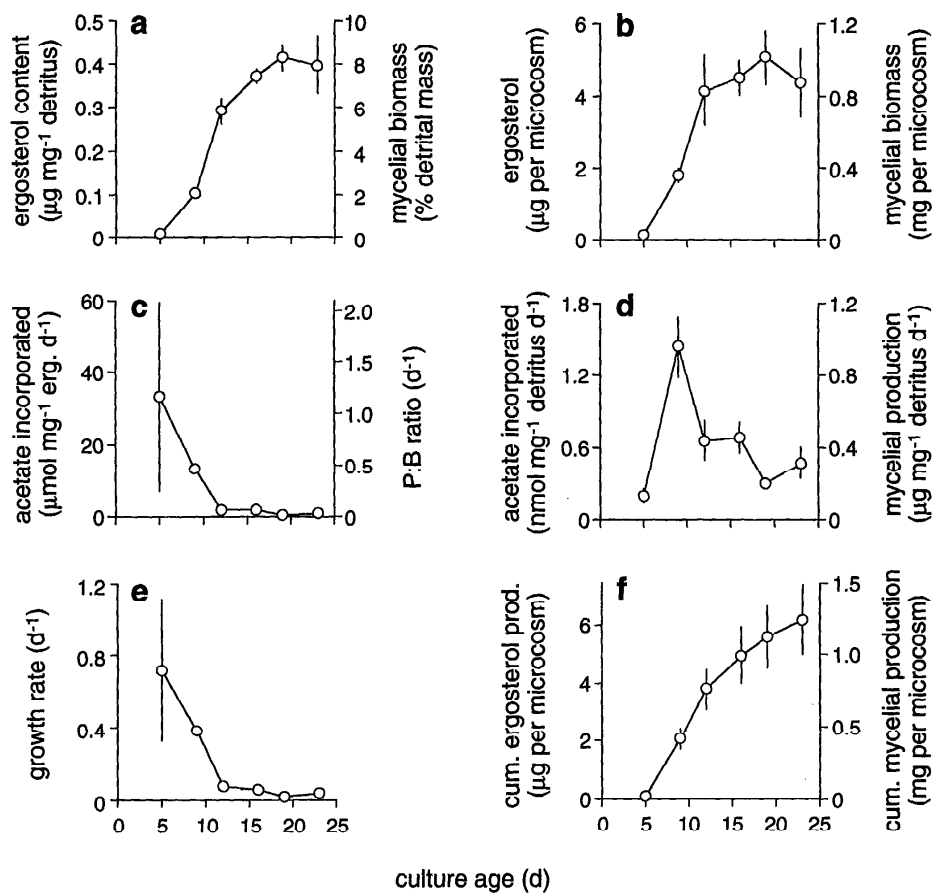


Fig. 6. Concentration (a) and amount (b) of mycelial biomass, specific production rate, or P:B ratio, (c), production rate (d), virtually instantaneous growth rate, μ (e), and cumulative production of *A. tetracladia* growing in ash leaf litter in stream microcosms. Biomass and production values were derived from measurements of ergosterol, ergosterol concentration of *A. tetracladia* of 5.0 mg g^{-1} mycelial dry mass (Gessner and Chauvet 1993), measured acetate incorporation rates, and a theoretical conversion factor of $6.6 \text{ mg mycelial dry mass per } \mu\text{mol incorporated acetate}$. Error bars indicate ± 1 SD of three replicate measurements, except for panel f where they indicate ranges.

sporulation data in a format similar to that of mycelial biomass (Fig. 6b), was likewise delayed.

During the 23 d of fungal growth in microcosms, ash leaves lost 58% of their initial dry mass (Fig. 8), a substantial portion of which (up to 22% of the initial mass) was probably lost due to leaching by day 5. Mass loss of leaves proceeded in an exponential fashion (Fig. 8) with nonlinear regression analysis yielding a breakdown coefficient of $0.045 \pm 0.003 \text{ d}^{-1}$ (mean $\pm 95\%$ C.I.).

Discussion

Methodology—As with analogous tracer techniques for planktonic microalgae and bacteria (Hurst et al. 1996), two basic prerequisites must be met to ensure a meaningful interpretation of acetate incorporation rates into ergosterol in terms of fungal growth and productivity. First, the time course of precursor incorporation into the target molecule

(ergosterol in the present case) must be linear during the incubation period. Deviation from linearity may indicate an adaptation of the fungus to the experimental situation, i.e. a metabolic switch resulting in estimates of growth rates and productivity different from those occurring in the natural habitat. The second prerequisite is that concentrations of added acetate during incubation are at saturation level so as to ensure maximization of precursor incorporation into the target endproduct. Fungal synthesis of ergosterol at the expense of carbon sources other than the added precursor would obviously lead to an underestimation of growth rate and productivity.

Under the conditions chosen in the present study, both requirements were met in three species of aquatic hyphomycetes growing in leaf litter in stream microcosms: linearity of precursor incorporation occurred for at least 10 h (Fig. 1) and saturation of incorporation rates was reached at an added acetate concentration in the lower millimolar range

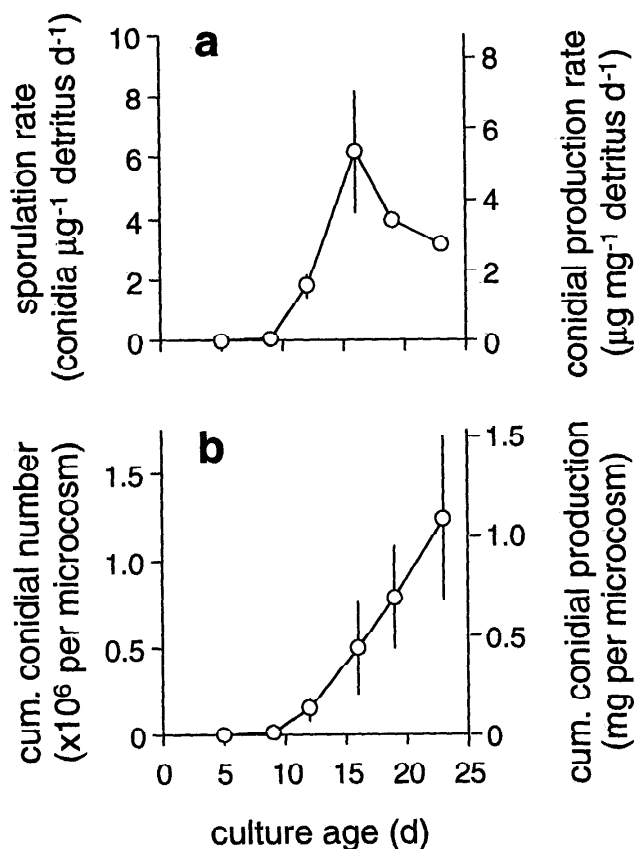


Fig. 7. Rates of sporulation and conidial production (a) and cumulative conidial output (b) of *A. tetracladia* growing in ash leaf litter in stream microcosms. Error bars indicate ± 1 SD of three replicate measurements (a) or ranges (b).

(Fig. 4). In addition, isotope dilution was found to be negligible (Fig. 5); the measured acetate incorporation rates can therefore be directly converted to fungal growth rates and productivities, provided that appropriate conversion factors are available (*see below*). Moreover, when experiments were carried out with leaf litter naturally colonized in a mountain stream, acetate incorporation was linear for 6 h. These findings are consistent with results on saprotrophic marine fungi reported by Newell and Fallon (1991), who found constant acetate incorporation rates over a period of 4 h and saturation of incorporation at an added acetate concentration of about 2 mM. Likewise, Suberkropp and Weyers (1996) in a field study on fungal productivity during leaf decomposition in streams obtained constant incorporation rates of acetate into ergosterol over at least 3 h and saturation at an added precursor concentration of about 5 mM. Thus, the acetate-to-ergosterol method appears to be a suitable technique to determine the productivity of fungi associated with decomposing leaf litter in aquatic ecosystems.

Time-course experiments with pure cultures of aquatic hyphomycetes in microcosms suggest that a lag-phase occurred before fungi began to incorporate acetate at a constant rate (Fig. 1). This phenomenon was not observed in similar stud-

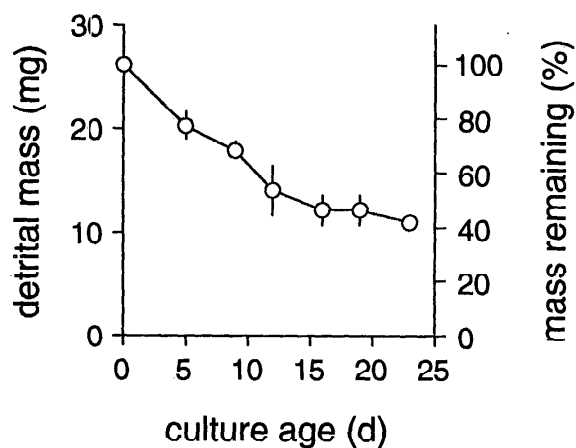


Fig. 8. Loss in dry mass of ash leaf litter decomposing in stream microcosms. Error bars indicate ± 1 SD of three replicate measurements.

ies (Newell and Fallon 1991; Suberkropp and Weyers 1996). One possible explanation is that in our study fungi were unable to assimilate the externally supplied acetate immediately. As this might indicate a fungal adaptation to the experimental situation, the possibility that our measured acetate incorporation rates did not reflect natural growth cannot be entirely ruled out. However, an alternative explanation is that diffusion of the precursor to the uptake sites within the leaf tissue was physically impaired (though samples were gently agitated during incubations). In that case, the slope of regression lines of elapsed time versus acetate incorporation would reflect accurate growth rates, and incorporations measured over relatively long incubation periods (e.g. 6 h) might provide reasonable estimates as well.

In contrast to the microcosm experiments, linearity of acetate incorporation with field-collected material occurred only for the first 6 h of incubation (Fig. 2). The subsequent deviation from linearity might again indicate that fungal metabolism became adapted to the experimental condition and growth was either limited (Fig. 2, top panels: stream water incubation) or stimulated (Fig. 2, bottom panels: culture medium incubation). Therefore, despite the linear incorporation in microcosms for 10 h, caution needs to be exercised when prolonged incubations are envisaged. We feel, however, that the potential shortcomings addressed above should not be overemphasized, given the limited data available to date, the fact that regression lines calculated for incorporation rates on an ergosterol basis (Fig. 2, right panels) were not significantly different, and that in other investigations (Newell and Fallon 1991; Suberkropp and Weyers 1996) similar observations were not made. Clearly, more studies in diverse systems are needed to put the acetate-to-ergosterol method on a firm basis.

Conversion factors—There are two possible approaches to obtain conversion factors relating acetate incorporation rates to fungal growth rate and production. The first approach takes advantage of existing biochemical knowledge about ergosterol biosynthesis in fungi. Although there are

Table 3. Maximum $P:B$ ratios (=specific production rates) of fungi associated with leaf litter in aquatic ecosystems.

System	Leaf species	Fungal assemblage	$P:B$ ratio (d^{-1})	Reference
Stream microcosm	Ash	<i>Articulospora tetracladia</i>	0.72*	Present study
Stream microcosm	Ash	<i>Lunulospora curvula</i>	0.11*	Present study
Stream microcosm	Ash	<i>Tetracladium marchalianum</i>	0.38*	Present study
Mountain stream	Ash	Aquatic hyphomycetes	0.017*	Present study
Lowland streams	Yellow poplar	Aquatic hyphomycetes	0.22†	Suberkropp 1995
Salt marsh	Smooth cordgrass	Marine ascomycetes	0.047†	Newell and Fallon 1991
Freshwater marsh	Sedge	Ascomycetes?	0.06†	Newell et al. 1995

* Based on theoretical conversion factor of 33 μg ergosterol μmol^{-1} incorporated acetate.

† Based on empirical conversion factors.

several alternative metabolic pathways leading to ergosterol in fungi, all result in the incorporation of 12 carbon atoms derived from the radiolabeled carboxyl group (C-1) of the acetate molecule (Weete 1989). The amount of ergosterol produced during incubations with radiolabel can therefore be directly derived from the number of incorporated acetate molecules. Further, if the proportion of ergosterol in fungal mass is known, the amount of mycelial biomass produced can be readily calculated. Given a total of 28 carbon atoms in ergosterol (molecular weight = 396.6), 1 μmol of incorporated acetate would thus correspond to 1/12 μmol ergosterol, which is equivalent to 33.05 μg . In *A. tetracladia* with a mycelial ergosterol concentration of 5.0 mg g^{-1} dry mass (Gessner and Chauvet 1993) this translates to a conversion factor of 6.61 $\text{mg mycelial biomass } \mu\text{mol}^{-1}$ of incorporated acetate.

An alternative approach to determine conversion factors consists in measuring acetate incorporation rates at one or several time points during early exponential growth when losses of fungal mass can be assumed to be insignificant and relate them to the changes in mycelial biomass occurring over the considered time interval. Assuming exponential growth of *A. tetracladia* in microcosms during initial growth (the first 5–12 d) (Fig. 6b), we calculate (by nonlinear regression analysis), an instantaneous growth rate (μ) of 0.322 $\text{mg ergosterol per mg ergosterol per day}$ (equivalent to a specific production rate or $P:B$ ratio of 0.324 d^{-1}). At day 9 the fungus incorporated 1.485 μmol acetate per mg ergosterol during the 3-h incubation period, and this results in a conversion factor from acetate (in μmol) to fungal mass (in mg) of 5.53 if the mycelial ergosterol concentration is 5.0 mg g^{-1} dry mass. Thus, our conversion factors derived from theoretical considerations (6.6 $\text{mg } \mu\text{mol}^{-1}$) and empirical measurements (5.5 $\text{mg } \mu\text{mol}^{-1}$) compare favorably, and this explains the close correspondence between the dynamics of fungal biomass (Fig. 6b) and estimates of cumulative mycelial production (Fig. 6f).

Newell and Fallon (1991) related acetate incorporation rates directly to changes in mycelial dry mass of two marine ascomycetes grown in liquid culture. The average conversion factors thus obtained (7.0 and 9.3 $\text{mg fungal organic mass per } \mu\text{mol acetate incorporated}$) were similar to the factors determined in the present study. Suberkropp and Weyers (1996), in contrast, reported empirically determined conversion factors for three species of aquatic hyphomycetes that were considerably higher (range of 17.6–21.0 $\text{mg } \mu\text{mol}^{-1}$) than the factors obtained for *A. tetracladia* in the present

study. Whether this discrepancy is caused by differences in fungal species or experimental conditions between the two studies (e.g. the use of the thin, soft-textured ash leaves versus the tougher leaves of yellow poplar, *Liriodendron tulipifera* L.) is currently unknown. Whatever the reason, it is worth noting that the empirical conversion factors in all three studies carried out to date are on the same order of magnitude. This suggests that the acetate-to-ergosterol technique yields reasonable estimates of fungal productivity associated with leaf litter decomposing in aquatic environments.

Fungal growth in stream-incubated leaf litter—Using the theoretical conversion factor deduced above, we calculated the apparent specific production rate ($P:B$ ratio) of the fungi associated with ash leaves that had been decomposing in a stream for 11 d as 0.017 d^{-1} (incubation in stream water; Table 3); together with an ergosterol concentration of 77.1 $\mu\text{g g}^{-1}$ leaf litter (Fig. 3b) and a mycelial ergosterol concentration of 5.5 mg g^{-1} (Gessner and Chauvet 1993), this results in a daily fungal production of 0.24 mg g^{-1} detrital mass. The maximum rates obtained in both microcosms and other aquatic ecosystems were much higher than the specific production rate realized in the present field study (Table 3), indicating that the potential for fungal growth in leaves is considerably higher than suggested by our stream data. Limited nutrient availability (Suberkropp 1995; Suberkropp and Chauvet 1995) is one of the possible explanations accounting for this phenomenon. This hypothesis is supported by the observation that fungal growth in field-collected leaf material was stimulated after 6 h when it was incubated in nutrient-rich culture medium (Fig. 2), whereas incubation in nutrient-poor stream water (Table 1) resulted in a stagnation of fungal growth at that time (Fig. 2). In line with this idea, sporulation rates of aquatic hyphomycetes as well as ergosterol concentrations in microcosms also were significantly higher after incubation in culture medium than after stream water incubation (Fig. 3).

Leaf mass loss, growth and sporulation of A. tetracladia in stream microcosms—The dynamics of leaf mass loss (Fig. 8), mycelial biomass (Fig. 6a), and sporulation rate (Fig. 7a) of *A. tetracladia* in microcosms were similar to the patterns observed in a previous field study with ash leaf litter decomposing in a mountain stream (Gessner and Chauvet 1994). Leaf breakdown coefficients (0.045 and 0.052 d^{-1} , respectively) and maximum sporulation rates (6.2 and 7.5 $\text{conidia } \mu\text{g}^{-1}$ detrital dry mass d^{-1}) were also similar, indicating that

Table 4. Production of *A. tetracladia* and potential fungal contribution to the mass loss of ash leaves in a stream microcosm.

Parameter	Mean	Range
Initial leaf mass (mg)	26.1	22.9–29.9
Leaf mass remaining at day 23 (mg)	10.9	10.3–11.5
Leaf mass loss (%)	58.1	55.9–60.5
Cumulative mycelial production (mg)	1.24	0.99–1.48
Cumulative conidial production (mg)	1.07	0.67–1.47
Total fungal production (mg)	2.31	1.66–2.95
Percentage of initial leaf mass assimilated (%)	25.3*	18.2–32.3
Fungal contribution to leaf mass loss (%)	43.5†	30.0–57.8

* Calculated by dividing total fungal production by an average fungal growth efficiency of 0.35 (Suberkropp 1991) and the average initial leaf mass.

† Estimated by dividing fungal assimilation by the loss in leaf mass.

the microcosm system mimicked natural conditions reasonably well. Note, however, that the two systems also differed in important aspects: both temperature and nutrient concentrations were much lower in the stream (Table 1), *A. tetracladia*, albeit present, was not the dominant fungus associated with leaves decomposing in the stream (Fig. 3a; see also Gessner et al. 1993), and maximum mycelial biomass was nearly twice as high in that natural system (155 mg g⁻¹ detrital dry mass; Gessner and Chauvet 1994).

Suberkropp (1991) calculated organic matter budgets for two species of aquatic hyphomycetes (*Anguillospora filiformis* Greathead and *L. curvula* Ingold) growing in yellow poplar leaves in stream microcosms. These budgets were based on measurements of leaf mass loss, mycelial biomass (derived from ATP determinations), conidial production and fungal respiration. After 1 month when 48% (*A. filiformis*) and 61% (*L. curvula*) of the initial leaf mass was lost, the two fungi had allocated 46% and 81%, respectively, of their total production to conidia. The estimated contribution of the fungi to total leaf mass loss over this period was 63 and 38% (calculated from table 3 in Suberkropp [1991]). Our estimates for *A. tetracladia* growing in ash leaves are consistent with these results: after 23 d, the fungus had allocated 46% of its total production to conidia, and it accounted for 44% of the total leaf mass loss (Table 4). Similar calculations of fungal contribution to mass loss of leaf litter decomposing in streams yielded estimates ranging from 31 to 66% (Baldy et al. 1995; Suberkropp 1995). Taken together, these results thus underline previous conclusions that fungi are eminently important agents of leaf decomposition in streams (Gessner et al. 1997) and transfer of allochthonous organic matter to higher trophic levels (Suberkropp 1992b).

The magnitude and dynamics of cumulative mycelial production (Fig. 6f) closely resembled those of mycelial biomass until about day 19 (Fig. 6b). This finding suggests that losses in mycelial biomass were small in the early growth phase. The steeper increase of the mycelial biomass between day 9 and 12 (Fig. 6b) compared with the cumulative mycelial production (Fig. 6f) might indicate that the acetate-to-ergosterol method slightly underestimated fungal production. Recall in this context that the conversion factor relating ac-

etate incorporation rate and mycelial production of *A. tetracladia*, and used for calculation of the values shown in Fig. 6d and f, was three times lower than the factors determined by Suberkropp and Weyers (1996) for three other species of aquatic hyphomycetes. Between day 19 and 23, the patterns of mycelial biomass (Fig. 6b) and cumulative mycelial production deviated from one another: average mycelial biomass decreased at this time, whereas cumulative fungal production increased further (Fig. 6f) at a relative rate similar to those measured during the preceding 10 d (Fig. 6d). Sporulation also continued at day 23 (Fig. 7) when about 40% of the initial leaf mass remained (Fig. 8). Thus, significant fungal production occurred at this stage. This production would go undetected if, as was done in the past, only fungal biomass and sporulation were measured in decomposing leaves. Clearly, the dynamics of fungal growth need to be taken into account fully to assess the fungal contribution to leaf decomposition in a realistic manner.

Two striking life history traits of aquatic hyphomycetes are that the sporulation maximum on decomposing leaves generally occurs before the maximum mycelial biomass is reached, and that changes in sporulation rate are more pronounced than corresponding changes in mycelial biomass (Suberkropp 1991; Baldy et al. 1995; Suberkropp and Chauvet 1995). That these are typical features of aquatic hyphomycetes is further corroborated by the present microcosm study (Figs. 6a and 7a). Mycelial growth rate of *A. tetracladia*, like sporulation rate, also was highest shortly after leaf occupation and subsequently dropped sharply (Figs. 6e, 7a). The maximum growth rate was even attained well before the maximum in sporulation rate occurred. Suberkropp (1995) observed exactly the same pattern in a field study on the productivity of fungi associated with decomposing leaves in lowland streams. Thus, one of the key features of aquatic hyphomycetes permitting these fungi to succeed in the stream environment appears to be their capacity to occupy and exploit available leaf resources rapidly, coupled with the allocation of resources to reproductive structures very early in their life cycle. This would be a profitable strategy indeed because the major energy source in temperate streams is generally available only during short periods of leaf fall and, although efficiently retained under conditions of base flow, is for the most part exported to downstream reaches during periods of high flow with the exact timing of these events being unpredictable.

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Received: 15 November 1995

Accepted: 14 May 1996

Amended: 7 January 1997