APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 2001, p. 5063–5068 Vol. 67, No. 11
0099-2240/01/$04.00+0 DOI: 10.1128/AEM.67.11.5063–5068.2001

Fungal Growth, Production, and Sporulation during Leaf Decomposition in Two Streams

KELLER SUBERKROPP*

Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama 35487-0206

Received 19 June 2001/Accepted 20 August 2001

I examined the activity of fungi associated with yellow poplar (*Liriodendron tulipifera*) and white oak (*Quercus alba*) leaves in two streams that differed in pH and alkalinity (a hardwater stream [pH 8.0] and a softwater stream [pH 6.7]) and contained low concentrations of dissolved nitrogen (<35 μg liter^{-1}) and phosphorus (<3 μg liter^{-1}). The leaves of each species decomposed faster in the hardwater stream (decomposition rates, 0.010 and 0.007 day^{-1} for yellow poplar and oak, respectively) than in the softwater stream (decomposition rates, 0.005 and 0.004 day^{-1} for yellow poplar and oak, respectively). However, within each stream, the rates of decomposition of the leaves of the two species were not significantly different. During the decomposition of leaves, the fungal biomasses determined from ergosterol concentrations, the production rates determined from rates of incorporation of [^{14}C]acetate into ergosterol, and the sporulation rates associated with leaves were dynamic, typically increasing to maxima and then declining. The maximum rates of fungal production and sporulation associated with yellow poplar leaves were greater than the corresponding rates associated with white oak leaves in the hardwater stream but not in the softwater stream. The maximum rates of fungal production associated with the leaves of the two species were higher in the hardwater stream (5.8 mg g^{-1} day^{-1} on yellow poplar leaves and 3.1 mg g^{-1} day^{-1} on oak leaves) than in the softwater stream (1.6 mg g^{-1} day^{-1} on yellow poplar leaves and 0.9 mg g^{-1} day^{-1} on oak leaves), suggesting that effects of water chemistry other than the N and P concentrations, such as pH or alkalinity, may be important in regulating fungal activity in streams. In contrast, the amount of fungal biomass (as determined from ergosterol concentrations) on yellow poplar leaves was greater in the softwater stream (12.8% of detrital mass) than in the hardwater stream (9.6% of detrital mass). This appeared to be due to the decreased amount of fungal biomass that was converted to conidia and released from the leaf detritus in the softwater stream.

Deciduous leaf litter is an important energy source for the food webs in woodland streams (7). However, leaf litter contains a number of plant polymers that are not easily digested by animal consumers, so microbial colonization and degradation are necessary to transform such detritus into a suitable food source for detritivores (3). Both fungi and bacteria colonize leaf litter after it enters streams and are involved in its decomposition. The biomass of fungi associated with leaf detritus is generally much higher than that of bacteria throughout decomposition (1, 31), and in culture fungi cause changes in leaf litter that are similar to those observed in streams (22). These observations suggest that fungi are the primary decomposers of leaves in streams. Some fungi also transform leaf litter into a more palatable and nutritious food source for invertebrate detritivores, indicating that fungi are also important intermediaries of energy flow between the leaf detritus and higher trophic levels (3).

The production of fungal biomass associated with decomposing leaf litter has been difficult to measure since the somatic hyphae of the fungi are immersed in the substrata that they are decomposing. The biomass of higher fungi growing in plant litter can be estimated from the concentrations of ergosterol, the major sterol associated with the cell membranes of the fungi (11). In conjunction with using ergosterol as a biomass index, Newell and Fallon (17) developed a method for estimating growth rates of litter-decomposing fungi from rates of incorporation of radiolabeled acetate into ergosterol. The production of fungal biomass can then be calculated by determining the product of growth rate and biomass. Both of these methods have been adapted for the fungi inhabiting streams (8, 10, 27).

Fungal activity associated with decomposing leaves is affected by the concentrations of the nutrients N and P dissolved in stream water (13, 21). The pH or alkalinity of the water also appears to affect fungal activity, but its effects have been difficult to interpret due to variations in the nutrient concentrations in the hardwater and softwater streams that have been examined (2, 23, 26, 31). My major objective in the present study was to compare fungal biomass and activity during leaf decomposition in two streams that differed in pH or alkalinity but contained similar concentrations of nutrients. I examined the activities of the fungi in a hardwater stream (pH 8.0) and a softwater stream (pH 6.7), both of which contained low concentrations of dissolved inorganic nitrogen (<35 μg liter^{-1}) and phosphorus (<3 μg liter^{-1}). I monitored fungal colonization of the leaves of two species, yellow poplar (*Liriodendron tulipifera*) and white oak (*Quercus alba*), to compare the dynamics of fungal growth and production in relation to decomposition rate. Fungal biomass associated with the leaves during decomposition was estimated from ergosterol concentrations, fungal growth rates were estimated from rates of incorporation of [^{14}C]acetate into ergosterol, and rates of production were calculated by determining the product of growth rate and biomass. As an additional index of fungal activity, the

* Mailing address: Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487-0206. Phone: (205) 348-1795. Fax: (205) 348-1403. E-mail: ksuberkp@biology.as.ua.edu.

TABLE 1. Rates of decomposition of leaves calculated per day (k) and per degree day (k')

Stream	Leaves	k (day^{-1})[a]	r^2	k' (degree day^{-1})[a]	r^2
Walker Branch	Yellow poplar	0.010 ± 0.0013 A[b]	0.89	0.00099 ± 0.00010 A	0.91
	White oak	0.007 ± 0.0006 A	0.93	0.00063 ± 0.00005 AB	0.94
Hugh White Creek	Yellow poplar	0.005 ± 0.0006 B	0.88	0.00079 ± 0.00099 AB	0.88
	White oak	0.004 ± 0.0004 B	0.86	0.00054 ± 0.00008 B	0.84

[a] Mean ± 95% confidence interval.
[b] Different letters indicate that differences between rates are significant ($P < 0.05$), as determined by analysis of covariance (Tukey's comparison).

rates of spore production associated with the leaves were determined.

MATERIALS AND METHODS

Study sites. The study was carried out in the west fork of Walker Branch (Anderson County, Tennessee; 35°58′N, 84°17′W), a second-order, spring-fed, hardwater stream (6, 16), and in Hugh White Creek (Macon County, North Carolina; 35°03′N, 83°26′W), a second-order, softwater stream (12, 28). The mean concentrations (ranges) of dissolved nutrients during the study in Walker Branch were as follows: ammonium N, 2 μg liter^{-1} (0 to 8 μg liter^{-1}); nitrate plus nitrite N, 18 μg liter^{-1} (4 to 28 μg liter^{-1}); and soluble reactive phosphorus, 2 μg liter^{-1} (1 to 3 μg liter^{-1}). The mean pH was pH 8.0, and the pH range was pH 7.8 to 8.2 (P. J. Mulholland, personal communication). The mean concentrations (ranges) of dissolved nutrients in Hugh White Creek were as follows: ammonium N, 2 μg liter^{-1} (1 to 4 μg liter^{-1}); nitrate plus nitrite N, 10 μg liter^{-1} (3 to 30 μg liter^{-1}); and soluble reactive phosphorus, <1 μg liter^{-1}. The mean pH was pH 6.7, and the pH range was pH 6.0 to 6.8 (J. Vose, personal communication). The mean daily temperature in each stream was calculated from data collected with temperature sensors (Ryan RTM 2000) which recorded the temperature at 30-min intervals.

Litter bags. Both yellow poplar leaves and white oak leaves were collected near Oak Ridge National Laboratory as they were naturally shed in the autumn, and they were air dried. Litter bags were made from fiberglass screening (1- by 1-mm mesh) into which two leaves of the same species were placed. For the litter bags that were to be used to determine decomposition rates, the leaves were weighed before they were placed in the bags. The litter bags were each attached to one of three plastic pipes that were placed on the bottom of each stream and anchored to concrete blocks. Litter bags were placed in Walker Branch on 7 November 1994 and in Hugh White Creek on 19 November 1994 and were removed at intervals thereafter. On each sampling date, litter bags containing leaves of each species were removed from each of the triplicate pipes in each stream and processed at the side of the stream. Leaves that had been preweighed were rinsed in stream water to remove debris and placed in containers on ice. These leaves were later dried at 60°C to a constant weight and ground with a Wiley mill, and a subsample of each sample was ignited at 500°C to determine the ash content. These leaves were used to determine the ash-free dry mass (AFDM) remaining for calculations of decomposition rates and nitrogen contents with a CHN analyzer (Carlos Erba). When leaves were placed in the streams, three litter bags for each species from each stream were dried, ashed, and weighed as described above to determine the factor used to convert the initial leaf air-dried weight to AFDM.

At each sampling time, triplicate litter bags for each species that had not been preweighed were also removed from the stream. The leaves were rinsed with stream water and subsampled at streamside by removing leaf disks (diameter, 11.6 mm) that were used for determinations of rates of incorporation of acetate into ergosterol, ergosterol concentrations, and sporulation rates. Five leaf disks were dried at 60°C and ignited at 500°C to determine the AFDM per disk in order to standardize other measurements on an AFDM basis.

Rates of incorporation of acetate into ergosterol. The growth rates of fungi associated with leaves were determined from acetate incorporation into ergosterol by the method proposed by Newell and Fallon (17) and modified for stream fungi (27). Five leaf disks from each replicate litter bag were placed in tubes containing 4.0 ml of filtered (0.45-μm-pore size membrane filters) stream water to which sodium [^{14}C]acetate (final specific activity, 48.5 MBq/mmol; ICN) was added to a final concentration of 5 mM. The tubes were incubated in a rack placed in the stream for 3 h with aeration (20 to 30 ml/min for each tube). Incubation was stopped by placing the tubes on ice and filtering leaf disks and particulate matter onto glass fiber filters (934/AH), which were placed in 5 ml of methanol, transported to the laboratory, and stored at −20°C until ergosterol was extracted. For the leaves of each species, the fungi in one tube were killed by adding formaldehyde (final concentration, 2%) and incubated with [^{14}C]acetate to determine the background levels of radiation associated with ergosterol.

Ergosterol was extracted from leaf disks by refluxing in alcoholic KOH (25 ml of methanol plus 5 ml of 4% KOH in 95% methanol) at 80°C for 30 min (18, 27). The leaf disks were removed, and the extract was transferred to tubes. Water (10 ml) and pentane (10 ml) were added, and the tubes were rotated for 3 min at 20 rpm with a mixer (Rotamix). The pentane fraction and two successive 5-ml aliquots of pentane mixed in the same way were then evaporated under a stream of N_2 at 30°C. The residue was dissolved in 1.0 ml of methanol and filtered (pore size, 0.45 μm; Acrodisc). Samples were later injected into a high-performance liquid chromatograph (Shimadzu) with a Whatman partisphere C_{18} column (length, 25 cm; diameter, 0.46 cm) by using methanol as the mobile phase (flow rate, 1 ml min^{-1}); the detector was set at 282 nm. A fraction collector (Advantec) that detected changes in the signal coming from the detector was used to collect ergosterol peaks. The radioactivity in the ergosterol fraction was determined for samples mixed with 10 ml of scintillation fluid (Ecolume) by using a scintillation counter (Beckman) that had been programmed to correct for quenching. The rates of incorporation of acetate into ergosterol were calculated from the radioactivity in the ergosterol fraction and were converted to instantaneous growth rates by multiplying by the empirically derived conversion factor determined for three aquatic hyphomycete species (19.5 μg of fungal biomass/nmol of acetate incorporated) (27). To convert the ergosterol content to fungal biomass, a factor of 5.5 mg of ergosterol/g of fungal biomass (8) was used. Fungal production (P) was calculated from $P = \mu B$, where μ is the instantaneous specific growth rate and B is the fungal biomass.

Sporulation rates. To determine sporulation rates, 10 leaf disks from each replicate were brought back to the laboratory and placed in 40 ml of filtered (0.45-μm-pore-size membrane filters) stream water in aeration chambers (22). The chambers were aerated with 100 ml of air/min for 24 h at 15°C, and aliquots of the water were filtered through membrane filters (5-μm-pore-size membrane filters, two filters for each replicate), fixed, and stained with 0.1% trypan blue in lactic acid. Conidia on the filters (25 fields at a magnification of ×160) were identified and counted.

Statistical analysis. Decomposition rates (k) were calculated by nonlinear regression (Systat for Windows, version 9) by using the model $m_t/m_0 = e^{-kt}$, where m_t is the AFDM remaining at time t (in days) and m_0 is the initial AFDM (5). To calculate decomposition rates on a degree day basis (k'), time t' (in degree days) was used. Differences in k determined from linear regressions of ln-transformed data were detected by analysis of covariance, followed by Tukey's comparison (32). Ergosterol contents, fungal production, and sporulation were examined within each stream by repeated measures analysis of variance (ANOVA), and these parameters were examined for types of leaves and streams by performing one-way ANOVA of maximum values. P values less than 0.05 were considered significant. Values are expressed below as means ± standard errors of the means.

RESULTS

Both types of leaves decomposed faster in Walker Branch than in Hugh White Creek ($P < 0.05$, as determined by analysis of covariance) (Table 1). In both streams, yellow poplar leaves decomposed at higher rates than white oak leaves, although the difference between species was not significant in either stream (Fig. 1 and Table 1). During the study, the mean daily temperatures of the water in Hugh White Creek were lower than those in Walker Branch (Fig. 2). The mean temperatures for the entire study were 7.0°C for Hugh White Creek and

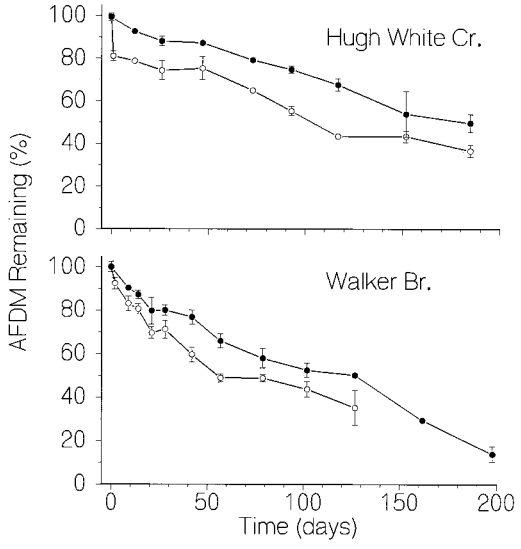

FIG. 1. AFDM of leaf detritus remaining in the two streams. Symbols: ●, means for white oak; ○, means for yellow poplar. The error bars indicate standard errors of the means.

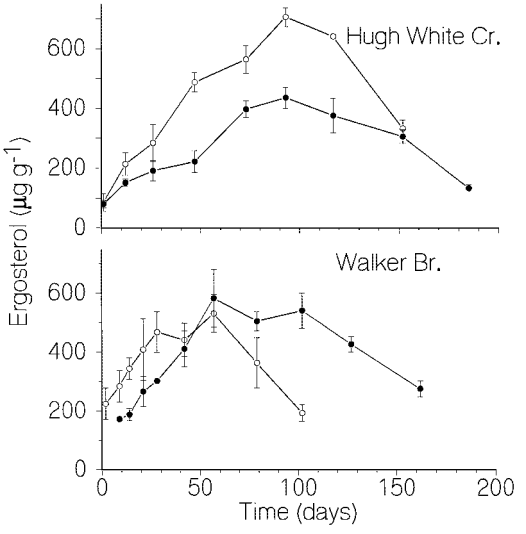

FIG. 3. Ergosterol concentrations (expressed in micrograms per gram of leaf detritus AFDM) associated with leaves during decomposition in the two streams. Symbols: ●, means for white oak; ○, means for yellow poplar. The error bars indicate standard errors of the means.

10.9°C for Walker Branch. When decomposition rates were calculated by using degree days to account for temperature differences, the rates of decomposition of the two types of leaves were higher in Walker Branch than in Hugh White Creek, but the difference between the streams for each species was not significant (Table 1).

In Hugh White Creek, the ergosterol concentrations for yellow poplar leaves increased faster than the ergosterol concentrations for white oak leaves (as determined by repeated measures ANOVA) (Fig. 3). In Walker Branch, the ergosterol concentrations associated with yellow poplar leaves increased without a lag and leveled off sooner than the ergosterol concentrations associated with white oak leaves. However, the maximum ergosterol concentrations for yellow poplar leaves were slightly lower than those for oak leaves in Walker Branch. For all leaves the maximum fungal biomass was ca. 10% of the total detrital mass (in Hugh White Creek, 12.8% of yellow poplar mass and 7.9% of white oak mass; in Walker Branch, 9.6% of yellow poplar mass and 10.6% of white oak mass), and the values were not significantly different. The ergosterol concentrations for both types of leaves reached maximum values sooner in Walker Branch (57 days) than in Hugh White Creek (93 days).

The initial nitrogen content of yellow poplar leaves (0.88%) was slightly higher than that of white oak leaves (0.77%). During decomposition, the nitrogen contents of all leaves increased (Fig. 4). The maximum nitrogen concentrations were higher in yellow poplar leaves (1.60% in Walker Branch, 1.76% in Hugh White Creek) than in white oak leaves (1.44% in Walker Branch, 1.26% in Hugh White Creek).

In Walker Branch, the rates of fungal production determined from rates of incorporation of [^{14}C]acetate into ergosterol and biomass were highest during the first 30 days that leaves were in the stream (Fig. 5) and then declined to low levels throughout the rest of the study. The rates of production remained relatively constant in Hugh White Creek. The fungal

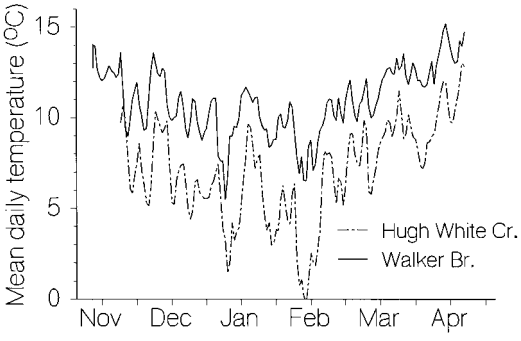

FIG. 2. Mean daily temperatures in the two streams.

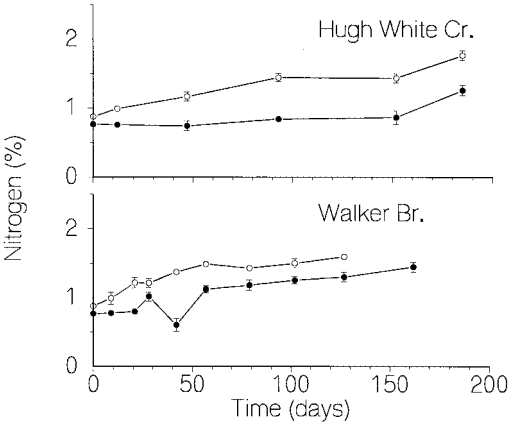

FIG. 4. Nitrogen concentrations (expressed as a percentage of leaf AFDM) associated with leaves during decomposition in the two streams. Symbols: ●, means for white oak; ○, means for yellow poplar. The error bars indicate standard errors of the means.

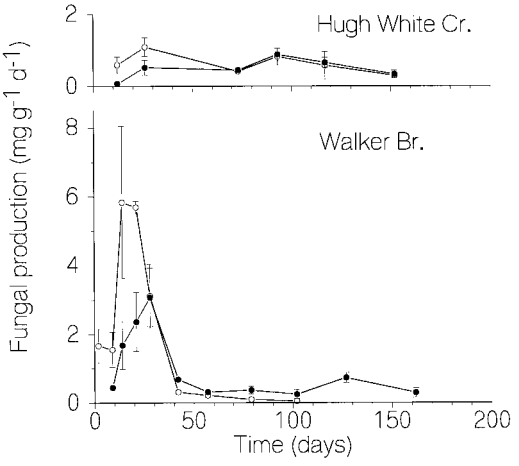

FIG. 5. Rates of fungal production (expressed in milligrams of fungus per gram of leaf detritus AFDM per day) associated with leaves during decomposition in the two streams. Symbols: ●, means for white oak; ○, means for yellow poplar. The error bars indicate standard errors of the means.

TABLE 2. Aquatic hyphomycete community compositions for leaves from the streams at the time when maximum sporulation rates occurred

Species	% of total conidia			
	Walker Branch		Hugh White Creek	
	Yellow poplar leaves	Oak leaves	Yellow poplar leaves	Oak leaves
Alatospora acuminata	13.4	2.8	20.4	5.5
Anguillospora filiformis	0	0	0	3.5
Anguillospora longissima	0.3	0	0	0
Articulospora tetracladia	0	0	43.7	42.1
Caesaria sphagnorum	0	0	0	0.9
Clavatospora tentacula	2.5	0.5	0	0
Clavariopsis aquatica	0	0.4	0	0
Flagellospora curvula	0	0	31.0	38.5
Heliscus lugdunensis	0	0	0	1.0
Lunulospora curvula	30.0	94.6	0	0
Tetrachaetum elegans	0	0	1.0	3.0
Tetracladium marchalianum	54.0	1.5	0	0
Tricladium chaetocladium	0	0	2.0	2.1
Other	0	0	1.9	3.5

production associated with yellow poplar leaves was significantly greater than that associated with white oak leaves in Walker Branch but not in Hugh White Creek during the first 30 days (as determined by repeated measures ANOVA). The maximum growth rates were higher in Walker Branch (9.4%/day on yellow poplar, 5.6%/day on white oak) than in Hugh White Creek (2.2%/day on yellow poplar, 1.4%/day on white oak), as determined by ANOVA.

The patterns of spore production also differed in the two streams (Fig. 6). The maximum rates of sporulation (per milligram of AFDM of leaves) were highest for yellow poplar leaves in Walker Branch after leaves had been in the stream for 21 to 28 days and were higher than the maximum rates of sporulation for all other treatments. The maximum rates of sporulation for white oak leaves in Walker Branch were approximately one-half those for yellow poplar leaves and occurred after the leaves had been in the stream for 40 to 60 days (Fig. 6). In Hugh White Creek, the maximum rates of sporulation for the two species were similar, were less than one-half the maximum rate of sporulation for oak leaves in Walker Branch, and occurred after the leaves had been in the stream for 93 days. Overall, the sporulation rates were not significantly different for the two leaf types in either stream (as determined by repeated measures ANOVA). When the sporulation rates were calculated on the basis of the fungal biomass (ergosterol concentrations) associated with the leaves, the sporulation rates for yellow poplar and white oak leaves were similar and both exhibited maxima after 21 days (960 and 1,080 spores μg of ergosterol^{-1} day^{-1}, respectively) (data not shown). The sporulation rates based on ergosterol concentrations through the first 73 days were also similar for the two types of leaves in Hugh White Creek. In this stream, the maximum rates of sporulation were only 100 and 180 spores μg of ergosterol^{-1} day^{-1} on yellow poplar and white oak leaves, respectively. Although the same fungal species sporulated on both yellow poplar and white oak leaves in Walker Branch, the species that was dominant varied (Table 2). On yellow poplar leaves, *Tetracladium marchalianum* was the dominant species, followed by *Lunulospora curvula*. On oak leaves, *L. curvula* accounted for more than 90% of the conidia produced. Other than *Alatospora acuminata*, which occurred in both streams, the species that occurred on leaves in Hugh White Creek differed from those that occurred on leaves in Walker Branch. *Articulospora tetracladia* and *Flagellospora curvula* were the dominant species on both types of leaves in this stream (Table 2).

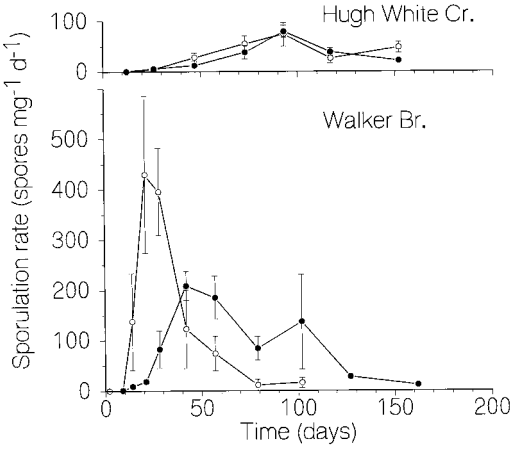

FIG. 6. Sporulation rates (expressed in number of spores produced per milligram of leaf detritus AFDM per day) associated with leaves during decomposition in the two streams. Symbols: ●, means for white oak; ○, means for yellow poplar. The error bars indicate standard errors of the means.

DISCUSSION

The breakdown rates which I determined for yellow poplar and white oak leaves are within the ranges that have been reported for these species in other studies (26, 30). White oak leaves typically decompose more slowly than yellow poplar leaves. I found this trend in both streams, but the rates of decomposition of the two types of leaves were not significantly

different. One reason for this may have been the presence of leaf-shredding invertebrates that invaded litter bags during the later stages of decomposition. Although I used a relatively fine-mesh screen (1 by 1 mm) to prevent invertebrates from consuming leaves, this strategy was not totally successful. In some litter bags, particularly late in the decomposition sequence, skeletonization of leaves indicative of invertebrate feeding was noted. Invertebrates (Trichoptera and Plecoptera in Hugh White Creek, Trichoptera and Amphipoda in Walker Branch) were also found in a few litter bags.

When yellow poplar leaves were placed in Hugh White Creek, they lost 19% of their AFDM in the first day (Fig. 1). This loss was presumably due to leaching; however, yellow poplar leaves in Walker Branch lost only 8% of their AFDM during the first 2 days that they were in the stream. Since the leaves placed in the two streams came from the same collection, this suggests that the lower pH of the water in Hugh White Creek resulted in greater losses due to leaching of leaves in this creek than in Walker Branch.

The acetate-to-ergosterol method for estimating fungal production has been used in studies of leaf decomposition in streams and appears to provide reasonable estimates of the rates of fungal production (10, 31). During the initial period of decomposition when fungi are colonizing leaf material and fungal biomass is increasing, growth rates calculated from changes in ergosterol content are correlated and show general agreement with growth rates determined from rates of incorporation of acetate into ergosterol (23, 25). In the present study, these two methods of determining growth rates also produced similar results. The ratios of the growth rates determined from changes in ergosterol concentrations during the initial periods of increase to the growth rates calculated from rates of incorporation of acetate into ergosterol over the same time periods were 0.5 (yellow poplar) and 0.9 (white oak) in Walker Branch and 1.7 (yellow poplar) and 3.4 (white oak) in Hugh White Creek (25). The lower values for Walker Branch appear to be due to the fact that in this stream more biomass was being converted into conidia that were released from the leaves, whereas in Hugh White Creek very little sporulation occurred during the period when the ergosterol content was increasing. In addition, the average temperature during the incubations with [^{14}C]acetate in Walker Branch (12.9°C) was higher than the average of the mean daily temperatures during this period (11.6°C), whereas the average incubation temperature in Hugh White Creek (5.5°C) was slightly lower than the average of the mean daily temperatures (6.3°C) for the corresponding period. These temperature differences should have contributed to the fact that the growth rates calculated from changes in ergosterol content were less than the growth rates determined from the rates of acetate incorporation in Walker Branch and greater than the growth rates determined from the rates of acetate incorporation in Hugh White Creek.

After leaves entered the streams, particularly Walker Branch, the changes in fungal activity were dynamic, and the levels of growth and activity reached maximum values early in the decomposition process. As leaves became more degraded, fungal biomass and activity (production and sporulation) declined. These patterns of changes in biomass and sporulation are similar to those found associated with leaves decomposing in other streams (9, 23, 26, 31). The maximum amounts of ergosterol associated with leaves in Walker Branch and Hugh White Creek (450 to 700 $\mu g\ g^{-1}$) are generally in the range found in other streams (400 to 900 $\mu g\ g^{-1}$), including streams with moderate to high concentrations of N and P (9, 23, 31). However, the maximum rates of fungal production associated with yellow poplar leaves in the present study (1.1 to 5.8 mg g^{-1} day^{-1}) are lower than those reported for other streams with moderate to high concentrations of nutrients in which this parameter has been measured (6.5 to 16 mg g^{-1} day^{-1}) (23, 31). The maximum sporulation rates (80 to 450 conidia mg^{-1} g^{-1}) are also generally lower than those reported for streams with moderate to high concentrations of N and P (100 to 6,000 conidia mg^{-1} g^{-1}) (9, 23, 26). Consequently, it appears that when aquatic hyphomycetes are provided with higher concentrations of N and P in the water, they exhibit higher levels of production and shunt more of the production into sporulation. Increased sporulation has also been observed in experiments in which nutrients were added to decomposing leaves in both field (13) and laboratory studies (21, 24).

Of the parameters measured in the present study, the rates of fungal production and sporulation differed the most when the values for the two streams were compared. Initially, both these activities were much higher in Walker Branch, the hardwater stream, than in Hugh White Creek, the softwater stream. The rates of production and sporulation for both types of leaves in Hugh White Creek increased later in the decomposition process and never reached values comparable to those obtained for Walker Branch. In both streams, however, increases in the rates of production preceded or coincided with increases in the rates of sporulation. It appears that a considerable fraction of the fungal production in Walker Branch was converted into conidia that were released from the leaves for downstream colonization. This would explain the observation that the ergosterol content of yellow poplar leaves in Walker Branch did not reach the levels observed for yellow poplar leaves in Hugh White Creek. In Walker Branch, a significant proportion of the biomass was released as conidia, and examination of changes in biomass as measured by ergosterol concentrations associated with the leaves would not have detected this extra production. Since fungal conidia are typically rich in proteins and nucleic acids, the greater rates of sporulation for leaves in Walker Branch also suggest that the amounts of total nitrogen associated with leaves in this stream would have been larger if the sporulation rates had been lower. Under certain conditions, sporulation can be a significant fate of biomass. For example, in cultures in which leaf material has been the sole carbon source, species of aquatic hyphomycetes have been found to allocate 44 to 81% of their total production to conidia (10, 22).

The differences in production and sporulation between the two streams were apparently not due to differences in the inorganic N and P dissolved in the water since both streams had very low concentrations of these nutrients. Even though the nutrient concentrations in Walker Branch were slightly higher than those in Hugh White Creek, both N and P have been shown to limit decomposition rates and fungal activity in Walker Branch (13). Hugh White Creek was cooler by an average of 4°C than Walker Branch, and this could explain why the activity in Hugh White Creek was somewhat lower than that in Walker Branch. The rates of decomposition of the

leaves were also lower in the softwater stream (Hugh White Creek) than in the hardwater stream (Walker Branch) and remained lower (but not significantly lower) when differences in temperature were taken into account by determining decomposition rates on a degree day basis. Another factor which could have contributed to differences in production and sporulation is the pH or alkalinity of the streams. Differences in pH or alkalinity affect the fungal species present (2, 26), and the streams examined in the present study contained species typical of soft- and hardwater streams; for example, *A. tetracladia* is common in softwater streams, *T. marchalianum* is common in hardwater streams, and *A. acuminata* is common in both types of streams. *L. curvula* can be found in both types of streams (26) but appears to be limited by cold temperatures, which may explain its absence from Hugh White Creek. It does not appear likely that these differences in species composition contributed to the differences in activity observed in the present study, since species isolated from hardwater and softwater streams exhibit similar activities when they are grown on leaves in culture (22). Differences in sporulation and production similar to those seen in the present study have been noted in comparisons of hardwater and softwater streams previously (23, 31), but variations in the N and P concentrations among streams also occurred and were thought to be primarily responsible for these differences. In culture, aquatic hyphomycetes grow at a broad range of pH values and are generally inhibited as the pH increases above neutral (19). However, in streams, pH has been shown to have variable effects on decomposition. In some studies, decomposition rates have been found to increase as the pH increases (4, 20, 29), whereas in other studies, the decomposition rates in streams at pH 5.9 to 6.2 have been found to be greater than the decomposition rates in streams with pH values greater than 7.5 (14, 15). Increased fungal activity is generally found in hardwater streams with a pH of 7.5 or higher in comparison to softwater streams with a pH of 7 or less (4, 20, 26). Data obtained in the present study support this conclusion and suggest that additional data are needed to more fully understand the effects of pH and alkalinity on the fungi colonizing leaves in streams.

ACKNOWLEDGMENTS

I thank V. Gulis for comments on the manuscript. I am grateful to P. J. Mulholland for allowing me to perform studies in his laboratory, for facilitating access to Oak Ridge National Laboratory and Walker Branch in the Oak Ridge National Environmental Research Park, and for providing water chemistry data for Walker Branch. I thank Wayne Swank for allowing me to perform studies in the Coweeta Hydrologic Laboratory of the U.S. Forest Service and James Voss for providing water chemistry data for Hugh White Creek.

This study was supported by grant DEB 9407232 from the National Science Foundation.

REFERENCES

1. **Baldy, V., M. O. Gessner, and E. Chauvet.** 1995. Bacteria, fungi and the breakdown of leaf litter in a large river. Oikos **74:**93–102.
2. **Bärlocher, F.** 1982. Conidium production from leaves and needles in four streams. Can. J. Bot. **60:**1487–1494.
3. **Bärlocher, F.** 1985. The role of fungi in the nutrition of stream invertebrates. Bot. J. Linn. Soc. **91:**83–94.
4. **Chamier, A.-C.** 1992. Water chemistry, p. 152–172. *In* F. Bärlocher (ed.), The ecology of aquatic hyphomycetes. Springer Verlag, Berlin, Germany.
5. **Chauvet, E.** 1987. Changes in the chemical composition of alder, poplar and willow leaves during decomposition in a river. Hydrobiology **148:**35–44.
6. **Elwood, J. W., and R. R. Turner.** 1989. Streams: water chemistry and ecology, p. 301–350. *In* D. W. Johnson and R. I. Van Hook (ed.), Analysis of biogeochemical cycling processes in Walker Branch watershed. Springer Verlag, New York, N.Y.
7. **Fisher, S. G., and G. E. Likens.** 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecol. Monogr. **43:**421–439.
8. **Gessner, M. O., and E. Chauvet.** 1993. Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. Appl. Environ. Microbiol. **59:**502–507.
9. **Gessner, M. O., and E. Chauvet.** 1994. Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology **75:**1807–1817.
10. **Gessner, M. O., and E. Chauvet.** 1997. Growth and production of aquatic hyphomycetes in decomposing leaf litter. Limnol. Oceanogr. **42:**496–505.
11. **Gessner, M. O., and S. Y. Newell.** 1997. Bulk quantitative methods for the examination of eukaryotic organoosmotrophs in plant litter, p. 295–308. *In* C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stetzenbach, and M. V. Walter (ed.), Manual of environmental microbiology. American Society for Microbiology, Washington, D.C.
12. **Golladay, S. W., J. R. Webster, and E. F. Benfield.** 1989. Changes in stream benthic organic matter following watershed disturbance. Holarct. Ecol. **12:** 96–105.
13. **Grattan, R. M., and K. Suberkropp.** 2001. Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. J. N. Am. Benthol. Soc. **20:**33–43.
14. **Griffith, M. B., and S. A. Perry.** 1994. Fungal biomass and leaf litter processing in streams of different water chemistry. Hydrobiology **294:**51–61.
15. **Griffith, M. B., S. A. Perry, and W. B. Perry.** 1995. Leaf litter processing and exoenzyme production on leaves in streams of different pH. Oecologia **102:** 460–466.
16. **Mulholland, P. J.** 1992. Regulation of nutrient concentrations in temperate forest stream: roles of upland, riparian, and instream processes. Limnol. Oceanogr. **37:**1512–1526.
17. **Newell, S. Y., and R. D. Fallon.** 1991. Toward a method for measuring instantaneous fungal growth rates in field samples. Ecology **72:**1547–1559.
18. **Newell, S. Y., T. L. Arsuffi, and R. D. Fallon.** 1988. Fundamental procedures for determining ergosterol content of decaying plant material by liquid chromatography. Appl. Environ. Microbiol. **54:**1876–1879.
19. **Rosset, J., and F. Bärlocher.** 1985. Aquatic hyphomycetes: influence of pH, Ca^{2+} and HCO_3^- on growth in vitro. Trans. Br. Mycol. Soc. **84:**137–145.
20. **Rosset, J., F. Bärlocher, and J. J. Oertli.** 1982. Decomposition of conifer needles and deciduous leaves in two Black Forest and two Swiss Jura streams. Int. Rev. Gesamten Hydrobiol. **67:**695–711.
21. **Sridhar, K. R., and F. Bärlocher.** 2000. Initial colonization, nutrient supply, and fungal activity on leaves decaying in streams. Appl. Environ. Microbiol. **66:**1114–1119.
22. **Suberkropp, K.** 1991. Relationships between growth and sporulation of aquatic hyphomycetes on decomposing leaf litter. Mycol. Res. **95:**843–850.
23. **Suberkropp, K.** 1995. The influence of nutrients on fungal growth, productivity, and sporulation during leaf breakdown in streams. Can. J. Bot. **73**(Suppl. 1)**:**S1361–S1369.
24. **Suberkropp, K.** 1998. Effect of dissolved nutrients on two aquatic hyphomycetes growing on leaf litter. Mycol. Res. **102:**998–1002.
25. **Suberkropp, K.** Estimating production of litter-decomposing fungi in streams from rates of acetate incorporation into ergosterol. Verh. Int. Verein. Limnol., in press.
26. **Suberkropp, K., and E. Chauvet.** 1995. Regulation of leaf breakdown by fungi in streams: influences of water chemistry. Ecology **76:**1433–1445.
27. **Suberkropp, K., and H. S. Weyers.** 1996. Application of fungal and bacterial production methodologies to decomposing leaves in streams. Appl. Environ. Microbiol. **62:**1610–1615.
28. **Swank, W. T., and J. B. Waide.** 1988. Characterization of baseline precipitation and stream chemistry and nutrient budgets for control watersheds, p. 57–79. *In* W. T. Swank and D. A. Crossley, Jr. (ed.), Forest hydrology and ecology at Coweeta. Springer Verlag, New York, N.Y.
29. **Thompson, P. L., and F. Bärlocher.** 1989. Effect of pH on leaf breakdown in streams and in the laboratory. J. N. Am. Benthol. Soc. **8:**203–210.
30. **Webster, J. R., and E. F. Benfield.** 1986. Vascular plant breakdown in freshwater ecosystems. Annu. Rev. Ecol. Syst. **17:**567–594.
31. **Weyers, H. S., and K. Suberkropp.** 1996. Fungal and bacterial production during the breakdown of yellow poplar leaves in 2 streams. J. N. Am. Benthol. Soc. **15:**408–420.
32. **Zar, J. H.** 1984. Biostatistical analysis, 2nd ed. Prentice Hall, Englewood Cliffs, N.J.