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Ergosterol-to-Biomass Conversion Factors for Aquatic Hyphomycetes

MARK O. GESSNER†* AND ERIC CHAUVET

Centre d'Ecologie des Ressources Renouvelables, Centre National de la Recherche Scientifique, 29 rue Jeanne Marvig, 31055 Toulouse, Cedex, France

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Fourteen strains of aquatic hyphomycete species that are common on decaying leaves in running waters were grown in liquid culture and analyzed for total ergosterol contents. Media included an aqueous extract from senescent alder leaves, a malt extract broth, and a glucose-mineral salt solution. Concentrations of ergosterol in fungal mycelium ranged from 2.3 to 11.5 mg/g of dry mass. The overall average was 5.5 mg/g. Differences among both species and growth media were highly significant but followed no systematic pattern. Stationary-phase mycelium had ergosterol contents 10 to 12% lower or higher than mycelium harvested during the growth phase, but these differences were only significant for one of four species examined. Availability of plant sterols in the growth medium had no clear effect on ergosterol concentrations in two species tested. To convert ergosterol contents determined in field samples to biomass values of aquatic hyphomycetes, a general multiplicative factor of 182 is proposed. More accurate estimates would be obtained with species-specific factors. Using these in combination with estimates of the proportion of the dominant species in a naturally established community on leaves resulted in biomass estimates that were typically 20% lower than those obtained with the general conversion factor. Improvements of estimates with species-specific factors may be limited, however, by intraspecific variability in fungal ergosterol content.

Much like forest floors, running waters meet a large portion of their carbon and energy requirements through allochthonous sources such as leaf litter derived from riparian trees. At base flow, this material is effectively retained in the channel (16) and rapidly broken down through biological and physical processes (4, 37). A conspicuous group of fungi that are able to sporulate under water, to grow at low temperature, and to produce the exoenzymes necessary for the degradation of structural polymers is fundamental to this process (32), owing to both their direct decomposer activity and their mediating role in the energy flow to higher trophic levels (3, 33).

A wealth of information has accumulated on the ecology of these aquatic hyphomycetes over the past 20 years (2), but remarkably little is known about the biomass and productivity of both these and other litter fungi: hence, their quantitative importance in the ecosystem. The main reason for this lies probably in the difficulty of separating, mechanically or optically, the intimate association between saprotrophic fungi and their substrates. With the advent of biochemical, immunological, and molecular biological techniques in ecology, however, this problem should now be overcome. A technique that is particularly promising in this respect consists in quantifying ergosterol, a cell constituent that is essentially restricted to the true fungi (21, 25). The application of this method has already led to new insights into a coastal marine detrital system (22) and to a preliminary appraisal of fungal importance for organic matter processing in streams (12, 14). It has also proven a useful tool for the study of a variety of other systems such as mycorrhizas (19, 31), soils (6, 39, 41), and coastal marine sediments (28).

Given the specificity of ergosterol (36, 38), its amount in a detrital particle such as a leaf is a relative measure of the fungal biomass associated with it. Knowledge of ergosterol concentrations alone, however, does not allow conclusions about the absolute amount of fungus present. For this, appropriate factors to convert ergosterol values into biomass in terms of mycelial dry mass (or similar parameters) are necessary. Such conversion factors can be established by isolating and cultivating the fungal species known to dominate the system under study and by measuring the ergosterol content in their mycelium. Matters are complicated by the fact that the concentration of a given cell constituent in an organism is not strictly constant but rather modulated by a host of internal and external factors. If valid conversion factors are to be obtained, then, culturing conditions must be chosen such that natural growth conditions are closely simulated. As for ergosterol, its amount in fungal tissue not only depends on the species (15, 23, 31) but also may vary with the physiological state of the fungus. It can thus depend on factors such as age, developmental stage (15), and general growth conditions (1). Safe (30) and Nout et al. (24), for example, found drastically reduced ergosterol concentrations in *Mucor rouxii* and *Rhizopus oligosporus* when these fungi were grown at low oxygen tensions (see also reference 7). This is not surprising because the metabolic pathway leading to ergosterol involves reactions that require molecular oxygen either directly (36) or via synthesis of heme components, which in turn are mandatory at several stages of sterol synthesis (18). In the typical habitat of aquatic hyphomycetes, i.e., turbulent headwater streams, O_2 concentrations can probably be assumed to be constantly high (although this might not be the case inside leaves), but other factors such as the chemical composition of the substrate (carbon source, C/N and C/P ratios, and concentration of specific inhibitors of ergosterol biosynthesis and other compounds reacting with ergosterol), temperature, and pH may similarly influence the lipid metabolism of fungi (38) and the ergosterol concentration in their membranes (1, 26, 36).

* Corresponding author.
† Present address: Projektzentrum Ökosystemforschung, Universität Kiel, Schauenburger Straße 112, 2300 Kiel 1, Germany.

TABLE 1. Composition of culture media

Medium	Carbohydrates (g/liter)	Carbon (g/liter)	Nitrogen (mg/liter)	Phosphorus (mg/liter)	C/N (g/g)	C/P (g/g)	pH
GMS	5.0	2.0	139	209	14	10	6.5
MEB	20.0[a]	8.4[a]	169		49		5.0
LEB	3.3[b]	3.6	32	15	113[c]	243[c]	5.0

[a] Assuming 100% maltose in malt extract.
[b] Glucose equivalents; phenol-sulfuric acid method.
[c] Lower when considering carbohydrate carbon only.

The aim of the present study was thus to determine ergosterol concentrations in the mycelium of aquatic hyphomycete species that are dominant members of the fungal assemblage on decaying leaves in running waters. While designing experiments, we had a double goal: (i) to establish empirical factors for the conversion of ergosterol to biomass under conditions that resemble natural ones, and (ii) to compare these with factors obtained under conditions that intentionally deviated from natural ones so as to gain insight into the magnitude of variation and hence the potential error when calculating fungal biomass from ergosterol concentrations measured in naturally decaying litter.

MATERIALS AND METHODS

Fungal isolates. Single spore isolates of aquatic hyphomycetes were obtained from leaf or foam samples taken from a variety of streams in southwestern France (5). Isolates included two strains of *Alatospora acuminata* Ingold (isolate 1, CERR 28.46; isolate 2, CERR 28.52), *Anguillospora longissima* (Sacc. & Syd.) Ingold (CERR 30.54), *Articulospora tetracladia* Ingold (CERR 29.73), *Clavariopsis aquatica* de Wildeman (CERR 28.55), *Clavatospora longibrachiata* (Ingold) Marvanová & S. Nilsson (CERR 28.56), two strains of *Flagellospora curvula* Ingold (isolate 1, CERR 28.82; isolate 2, CERR 30.67), *Lemonniera aquatica* de Wildeman (CERR 28.85), *Lemonniera terrestris* Tubaki (CERR 28.48), *Tetrachaetum elegans* Ingold (CERR 28.74), *Tetracladium marchalianum* de Wildeman (CERR 35.60), and *Tricladium splendens* Ingold (CERR 29.53). (Cultures can be obtained from E. Chauvet.) A culture of *Crucella subtilis* Marvanová was kindly provided by K. Suberkropp (Tuscaloosa, Ala.). All cultures were maintained on 2% malt agar (Merck) at 10°C until used in experiments.

Media. Three different media were used for liquid cultures: a mineral salt solution with glucose as the carbon source (GMS), a malt extract broth at 2% strength (MEB), and a leaf extract broth (LEB). GMS was similar to the media used by Suberkropp et al. (34) and Thornton (35) and consisted of 1 g of KNO_3, 0.65 g of KH_2PO_4, 0.35 g of $Na_2PO_4 \cdot 2H_2O$, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.15 g of $CaCl_2 \cdot 2H_2O$, 2 mg of $FeCl_3 \cdot 6H_2O$, 1 mg of $MnSO_4 \cdot H_2O$, 1 mg of $ZnSO_4 \cdot 7H_2O$, 1 mg of H_3BO_3, 0.1 mg of $AlSO_4 \cdot 18H_2O$, 0.1 mg of KI, 0.1 mg of $Na_2MoO_4 \cdot 2H_2O$, 25 μg of $CoCl_2 \cdot H_2O$, 25 μg of $NiCl_2 \cdot 6H_2O$, and 5 g of glucose in 1,000 ml of H_2O. In two experiments, GMS was supplemented with plant sterols (Sigma S-5753: 60% β-sitosterol, balance primarily campesterol) dissolved in 250 μl of ethanol, which was subsequently removed from the medium by brief heating. To provide a substrate that would approach conditions in nature, we also prepared a leaf extract by leaching 300 g of autumn-shed, air-dried, shredded alder (*Alnus glutinosa* (L.) Gaertn.) leaves in 15 liters of deionized water for 2 h at 10°C. The crude extract was filtered over Whatman GF/C filters, reduced to about 1 liter on a rotary evaporator, and freeze-dried. The final product was redissolved in stream water to give a stock solution and stored in portions at −18°C until needed. As required, stock solutions were diluted with stream water and characterized by using standard analytical procedures (10). Dissolved organic carbon was measured with a Dohrman TOC-Analyser (model DC-180). Media were autoclaved for 15 min at 121°C. Their chemical composition is summarized in Table 1.

Inoculum preparation and shake cultures. Four agar plugs of 8 mm in diameter were cut from the leading edge of a growing colony, submerged in 10 ml of sterile H_2O, and homogenized for 30 s, using an autoclaved Ultra-Turrax blender that was placed in a laminar flow cabinet. Two milliliters of the resulting homogenate was used to inoculate 100 ml of GMS in 500-ml flasks. If isolates would not grow on this medium (*A. acuminata* isolate 2 and *L. terrestris*), yeast extract was added at a concentration of 1 g/liter (34). These precultures were grown on a shaker at 15 or 20°C until peak biomass was reached (7 to 14 days depending on species) and homogenized. Two milliliters of the resulting suspension (corresponding to 8 to 10 mg of mycelial dry mass) was used to inoculate 250-ml culture flasks containing 50 ml of medium.

Three replicate flasks were randomly removed at each sampling data. The final pHs of the media were typically 8.4 for GMS, 7.5 for MEB, and 7.0 for LEB. The mycelium was collected on tared fiber glass filters (Whatman GF/C), washed twice with deionized water, and frozen at −18°C. It was then later freeze-dried and weighed to the nearest 0.1 mg. Mycelium used for ergosterol analyses was generally harvested during late growth (depending on species and medium, 6 to 10 days after inoculation), but mycelium of *C. longibrachiata* was collected only after 35 days owing to the slow growth of this fungus. For four strains, additional samples were taken during stationary phase (after 16 or 28 days; Fig. 1) to examine the effect of culture age on ergosterol contents in their mycelia.

Ergosterol analysis. Ergosterol was extracted from 50-mg

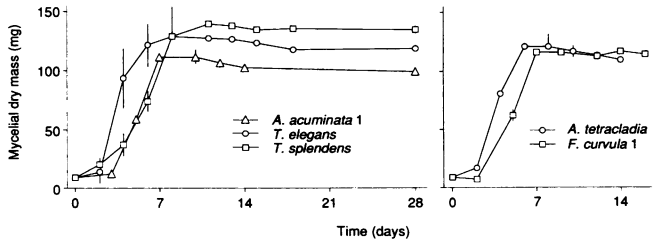

FIG. 1. Growth kinetics of aquatic hyphomycetes in shake culture. Incubation was in GMS at 15°C. Vertical bars denote ±1 standard deviation.

TABLE 2. Mycelial ergosterol content in aquatic hyphomycetes

Isolate	Ergosterol concn (mg/g of dry mass)[a]		
	GMS	MEB	LEB
A. acuminata 1	10.2 ± 0.9[b]	6.8 ± 0.4	7.3 ± 0.4
A. acuminata 2	6.5 ± 0.1		4.5 ± 0.1
Anguillospora longissima	4.2 ± 0.2		4.0 ± 0.3[c]
Articulospora tetracladia	4.2 ± 0.3	5.0 ± 0.1	
Clavariopsis aquatica	4.6 ± 0.1	4.2 ± 0.4	8.0 ± 0.3
Clavatospora longibrachiata	8.3 ± 0.5		
Crucella subtilis			3.3 ± 0.2
F. curvula 1	3.9 ± 0.3		
F. curvula 2	10.0 ± 0.5	11.5 ± 0.0	10.2 ± 0.0
Lemonniera aquatica	6.8 ± 1.1	2.3 ± 0.3	5.8 ± 0.5
Lemonniera terrestris	2.4 ± 0.1		5.3 ± 0.2
Tetrachaetum elegans	4.7 ± 0.4[b]	3.0 ± 0.1	2.9 ± 0.1
Tetracladium marchalianum	4.6 ± 0.6	3.3 ± 0.3	2.6 ± 0.4
Tricladium splendens	3.5 ± 0.3[b]		

[a] Mean ± 1 standard deviation.
[b] Average of three sets of cultures of varying age.
[c] Average of two sets of cultures of varying age.

samples by homogenization in methanol with an Ultra-Turrax blender (11). The extract was saponified by refluxing in alcoholic base, and lipids were partitioned into petroleum ether (bp, 35 to 60°C) by three successive washings with 20 ml of solvent each time. Samples were evaporated to dryness under a stream of nitrogen and then redissolved in 1 to 2 ml of methylene chloride. Ergosterol was quantified by using reverse-phase high-performance liquid chromatography (HPLC) with UV detection at 280 nm (11).

Statistical analysis. One- and two-way analyses of variance were used to test for effects of fungal species or strain, medium composition, and culture age on the ergosterol concentration in mycelia (40). Tukey's HSD test was used for posthoc pairwise comparisons when appropriate. The significance level was set at $P = 0.05$.

RESULTS

Ergosterol concentrations in mycelia ranged from 2.3 to 11.5 mg/g of dry mass (Table 2), with an overall average of 5.5 mg/g (Table 3). Differences among media were not significant when all data were analyzed together (Table 3). When considering each fungal strain separately, however, 73% of the pairwise comparisons resulted in significant differences between media, although there was no clear trend for higher or lower concentrations in one or the other medium (Table 2). Mycelium of *T. marchalianum*, for example, had the highest ergosterol content when grown in GMS and the lowest when grown in LEB, whereas *L. terrestris* showed exactly the opposite response; *F. curvula* isolate 2 had the lowest concentrations in MEB, while those of *L. aquatica* were highest in this medium. This phenomenon is reflected in the analysis of variance by the highly significant interaction term "species × medium" ($P < 0.0001$).

TABLE 3. Mean ergosterol content in mycelium of aquatic hyphomycetes

Medium	Mean (mg/g of dry mass)[a]	SD	Maximum/minimum	*n*
GMS	5.8	2.7	4.7	19
MEB	5.2	3.2	5.0	7
LEB	5.3	2.4	3.9	11
All media	5.5	2.6	5.0	37

[a] Differences are not significant ($P = 0.78$).

TABLE 4. Effect of culture age on mycelial ergosterol contents

Isolate	Medium	Culture age (days)	Ergosterol concn (mg/g of dry mass)[a]
Alatospora acuminata 1	GMS	7	10.1 ± 0.2[b]
		28	11.2 ± 0.1[b]
Anguillospora longissima	LEB	8	4.2 ± 0.2
		16	3.7 ± 0.0
Tetrachaetum elegans	GMS	8	5.0 ± 0.4
		28	4.5 ± 0.2
Tricladium splendens	GMS	8	3.4 ± 0.3
		11	3.5 ± 0.4
		28	3.8 ± 0.2

[a] Mean ± 1 standard deviation.
[b] Means are significantly different ($P < 0.01$).

Comparisons among species made separately for each medium produced clear differences in every case ($P < 0.0001$), with Tukey's HSD test resulting in significant differences in 75% of the pairwise combinations.

Table 4 shows the influence of culture age on mycelial ergosterol contents of four species. With the exception of *A. acuminata* isolate 1, the small differences were not significant. In addition, depending on fungal species, either the younger or the older mycelium had higher ergosterol contents, an observation confirmed by the significant interaction between fungal species and culture age.

Table 5 reveals that the ergosterol concentrations in two strains each of *A. acuminata* and *F. curvula* differed considerably. This was unlikely to be due to possible differences in experimental procedures because for both *A. acuminata* isolate 1 and *T. elegans* results were reproducible in two independent experiments (Table 5).

The addition of plant sterols to the culture medium did not affect ergosterol concentrations of *T. elegans* but resulted in a 15% reduction in *F. curvula* isolate 2 (Table 6). Mycelium from the control flask receiving ethanol without plant sterol

TABLE 5. Ergosterol content in different strains of two species and reproducibility of results in independent experiments

Isolate	Medium	Culture age (days)	Ergosterol concn (mg/g of dry mass)[a]
A. acuminata 1[b]	GMS	7	10.1 ± 0.2[c]
A. acuminata 1[b]	GMS	10	9.3 ± 0.9[d]
A. acuminata 2	GMS	9	6.5 ± 0.1[c,d]
A. acuminata 1	LEB	9	7.3 ± 0.4[e]
A. acuminata 2		9	4.5 ± 0.1[e]
F. curvula 1	GMS	7	3.9 ± 0.3[f]
F. curvula 2		8	10.0 ± 0.5[f]
T. elegans[b]	GMS	8	5.0 ± 0.4
T. elegans[b]	GMS	7	4.7 ± 0.6

[a] Mean ± 1 standard deviation.
[b] Independent runs.
[c,d,e,f] Values with identical superscript letters are significantly different ($P < 0.01$).

TABLE 6. Effect of plant sterols in standard medium (GMS) on ergosterol contents in aquatic hyphomycetes

Isolate	No. of analyses	Ergosterol concn (mg/g of dry mass)[a]	Comment
F. curvula 2	3	8.5 ± 0.7[b]	3.6 mg of plant sterol per liter
	1	7.7[b]	Control with ethanol
	3	10.0 ± 0.0[b]	No additives
T. elegans	2	5.1 ± 0.5	8 mg of plant sterol per liter[c]
	1	4.9	Control with ethanol
	3	4.7 ± 0.6	No additives

[a] Mean ± 1 standard deviation.
[b] Values are significantly different.
[c] Suspension.

showed even lower values, however, so this reduction cannot be clearly assigned to the availability of an external sterol source.

DISCUSSION

Choice of a general conversion factor. The mycelium of all aquatic hyphomycetes tested in the present study contained substantial amounts of ergosterol (Table 2). Concentrations are in general agreement with those determined for terrestrial and marine fungi (6, 19, 21, 24, 38), although some authors (27, 42) report values that are lower by as much as 1 order of magnitude.

With the highest value exceeding the lowest by a factor of 5 in the present study (Table 3), the ergosterol contents in aquatic hyphomycetes turned out to be reasonably uniform. This was so in spite of the contrasting composition of media (Table 1) and the rather high number of species examined. Differences of this magnitude are consistent with results reported for six saprotrophic salt-marsh fungi (23) and with values found in several mycorrhizal fungi (19, 31). Huang et al. (15), by contrast, reported much larger differences among macrofungal fruit bodies purchased at a local market, but these could have been due to varying amounts of fresh and old mycelia. Thus, it would appear that estimates of fungal biomass associated with leaf litter in streams can be made with reasonable accuracy by using a general conversion factor derived from the average ergosterol concentration in aquatic hyphomycetes.

This conclusion is further supported by the finding that ergosterol concentrations were generally little affected by culture age, both in the four aquatic hyphomycetes tested here (Table 4) and in a range of other fungi (6, 8, 15, 20, 31). It contrasts, however, with the results of Nout et al. (24), who found a marked decrease in the concentration of ergosterol in senescing cultures of *R. oligosporus*. That decrease was correlated with a rapid autolysis of mycelium after reaching peak biomass (see also reference 23), whereas in our experiments all strains maintained a high biomass during the extended stationary phase (Fig. 1). It would thus appear that ergosterol concentrations in aquatic hyphomycetes are largely independent of mycelial age and, owing to the rapid deterioration of the molecule upon cell death, indicative of live fungal biomass. This is a contention that has been expressed previously by West et al. (39) and Newell (21).

The average ergosterol content in all samples of the present study was 5.5 mg/g of fungal dry mass (Table 3); therefore, a general conversion factor of 182 (f = 1/5.5 · 1,000) is proposed for aquatic hyphomycetes. This factor is about 60% lower than those suggested previously for other systems (17, 20), but 34% higher than the one used by Newell et al. (22) for the salt-marsh fungus *Phaeosphaeria spartinicola*. That the factor of 182 proved to be quite robust toward the culture medium (f_{LEB} = 189; f_{GMS} = 172; and f_{MEB} = 192) in spite of marked differences in composition (Table 1) and significant amounts of phenolics in LEB (ca. 1.5 g/liter [10]) may be seen as evidence of its general applicability. When this factor is used with field samples, fungal biomass would, at worst, be underestimated by half (i.e., if the true ergosterol content of the fungi in the sample were 2.3 mg/g of dry mass) or overestimated by double (if the true concentration were 11.5 mg/g).

Species-specific conversion factors. The significant interaction term "fungal species × culture medium" in the analysis of variance suggests that the ergosterol content in aquatic hyphomycetes is dependent on both species and substrate quality. If so, species-specific conversion factors established under near-natural growth conditions together with information on the relative abundance of the dominant fungal species in the study system should improve estimates. Total fungal biomass (B) could then be calculated according to:

$$B = \Sigma E_i f_i \quad (1)$$

where E_i is the amount of ergosterol accounted for by species i, and f_i is the specific conversion factor from ergosterol to biomass for this species. E_i itself cannot be determined empirically but must be estimated as the product of the total amount of ergosterol (E) and the relative importance of species i in a given community (p_i). Equation 1 can then be rewritten as

$$B = E \Sigma p_i f_i \quad (2)$$

To compare estimates of fungal biomass in field samples made with the general and species-specific conversion factors, we introduced standard leaf packs into a mountain stream, subsequently retrieved them, and analyzed the species composition of the established hyphomycete community. The relative importance of species was determined by estimating the number of spores produced on disks punched out from leaves and incubated in the laboratory for 3 days. Details of procedures and a comprehensive account of the observed colonization patterns are given in Gessner et al. (13). The calculation of species-specific factors was based on ergosterol contents of cultures grown in leaf extract. When these data were not available, mean values over all media were used.

With generally 10 to 30% difference, the two estimation procedures produced similar results (Table 7). Apparently this was due to the high species diversity in samples (i.e., more that 10 frequent species [13]), which would dampen the impact of extreme values, and the fact that the general factor was derived from exactly those species dominating the fungal assemblages on leaves. Note, however, that with one exception higher estimates were always obtained with the general conversion factor. This effect was caused mainly by the high ergosterol content of *F. curvula* isolate 2 (10.2 mg/g of mycelial dry mass for the culture grown in leaf extract), since this species was predominant in all samples (13).

Problems. In the investigation by Newell et al. (23), different strains of two marine *Ascomycete* species had very similar ergosterol concentrations. By contrast, the ergosterol content of two isolates of *A. acuminata* and *F. curvula* examined here differed by factors of 1.5 and 2.5, respectively (Table 5). This is a dimension that is smaller than the

TABLE 7. Percent difference between estimates of fungal biomass on decaying alder leaves, using either general or species-specific conversion factors[a]

Season	% Difference between estimates at given colonization time		
	2 wk	4 wk	8 wk
Autumn	23 ± 7	−6 ± 15	10 ± 4
Winter	9 ± 2	14 ± 6	20 ± 1
Spring	30 ± 3	19 ± 7	
Summer	36 ± 2	18 ± 4	

[a] Positive values indicate that higher estimates were obtained with the general factor. Ergosterol concentrations ranged from 77 to 508 μg/g of detrital dry mass.

maximum difference among species (factor 5; Table 2) but great enough to lead to considerable error in the estimation of absolute biomass in naturally decomposing leaves. Therefore, intraspecific variability in ergosterol concentrations of fungi is a non-negligible phenomenon that needs further investigation.

To mimic natural conditions, the mycelia intended for ergosterol determinations were grown in a leaf extract prepared in stream water. This culture system differs nonetheless in two features from those growth conditions that aquatic hyphomycetes would normally encounter in nature. First, like other litter fungi, aquatic hyphomycetes grow generally within the leaf matrix where physical conditions differ markedly from those in liquid culture. Because the direct determination of fungal biomass inside a solid substrate is not possible, a reliable reference method to calibrate the ergosterol technique does, unfortunately, not exist. As a consequence, the potential disparity between mycelia grown in the field and those grown in the laboratory may remain a potential shortcoming for establishing reliable conversion factors, although in special cases the collection of sufficient amounts of fungal mycelium in nature may be possible (9).

Second, aqueous leaf extracts lack lipophilic leaf components, including sterols, whose presence in nature could affect the ergosterol content in fungi. Because most of the requirements of fungi for sterols appear to be rather unspecific (29, 36, 38), it is conceivable that plant sterols are incorporated into fungal membranes, where they would partially replace ergosterol. This would result in an underestimation of true fungal biomass in leaf litter. Conversely, the abundant supply of plant sterols and other lipids in leaves could prompt their utilization as metabolic precursors for ergosterol biosynthesis and therefore result in elevated ergosterol concentrations in saprotrophic fungi. This would mean an overestimation of true fungal biomass. To study these effects, Newell et al. (23) suggested complementing aqueous extracts of leaves with an alcoholic leaf extract and offering this mixture, after evaporation of the alcohol, as a culture medium. Another possibility is to add commercially available plant sterols to standard medium (Table 6). In both cases, however, the poor solubility of sterols in water allows the addition of only minute amounts unless the formation of a suspension is accepted. At an ergosterol concentration of 10 mg/g of mycelial dry mass for *F. curvula* isolate 2 (Table 6) and a mycelial production of about 2.5 g/liter of culture medium (Fig. 1), for example, the added plant sterol would cover only 15% of the culture's requirements for ergosterol. In the experiment with *T. elegans*, however, two-thirds of the need would have been met by added plant sterol (Table 6). That these "concentrations" did not result in significant changes in mycelial ergosterol contents indicates that they are little affected by the presence of external sterol. This finding reminds one of the phenomenon of "aerobic sterol exclusion" described for the yeast *Saccharomyces cerevisiae*, which under aerobic conditions makes no use of externally supplied sterols (18). Given that metabolic costs for de novo synthesis of steroids are considerable, the phenomenon would appear to be linked to functional properties of ergosterol (18). If so, the principle should also apply to aquatic hyphomycetes, but more studies are certainly needed before broad conclusions can be drawn.

Newell et al. (23) found unusually high ergosterol concentrations in *P. spartinicola* when this fungus was grown on its natural substrate, namely, leaves of *Spartina alterniflora*. Similar results were obtained by Nout et al. (24) when their fungus, *R. oligosporus*, was grown on soybeans. In both studies, however, reference values of biomass were derived from measurements of hyphal lengths. Although internally calibrated, this technique suffers from a number of systematic insufficiencies that probably result in a severe underestimation of biomass (9, 21), making the interpretation of mycelial ergosterol concentrations calculated on this basis questionable (23).

The determination of fungal biomass in ecological systems by the ergosterol technique is still in its infancy. Consequently, the conversion factors proposed here need to be critically evaluated. The degree of intraspecific variability, the effect of solid substrates, and the influence of plant sterols and other lipids on mycelial ergosterol concentrations deserve special attention in this context. In addition, comparisons with alternative methods such as enzyme-linked immunosorbent assay, radioimmunoassay, hexosamine assay, and ATP assay (9, 21) should be made with mycelium grown inside the leaf matrix.

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