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# Comparison of ATP and Ergosterol as Indicators of Fungal Biomass Associated with Decomposing Leaves in Streams

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ATP and ergosterol were compared as indicators of fungal biomass associated with leaves decomposing in laboratory microcosms and streams. In all studies, the sporulation rates of the fungi colonizing leaves were also determined to compare patterns of fungal reproductive activity with patterns of mycelial growth. During leaf degradation, ATP concentrations exhibited significant, positive correlations with ergosterol concentrations in the laboratory and when leaves had been air dried prior to being submerged in a stream. However, when freshly shed leaves were submerged in a stream, concentrations of ATP and ergosterol were negatively correlated during degradation. This appeared to be due to the persistence of leaf-derived ATP in freshly shed leaves during the first 1 to 2 weeks in the stream. Estimates of fungal biomass from ergosterol concentrations of leaf litter were one to three times those calculated from ATP concentrations. ATP, ergosterol, and sporulation data generally provided similar information about the fungi associated with decomposing leaves in streams during periods when fungi were growing. Ergosterol concentrations provide a more accurate indication of fungal biomass in situations in which other organisms make significant contributions to ATP pools.

Fungi, particularly aquatic hyphomycetes, are considered to be the primary decomposers of leaf litter in streams (3, 24). However, estimates of the amounts of fungal biomass associated with leaves colonized in streams vary considerably. This is due, in part, to differences in the amounts of biomass that can accumulate on leaves in streams with different physicochemical characteristics (21, 23); however, it also reflects differences in the methods that have been used to estimate biomass.

In streams, concentrations of fungal biomass have been estimated from microscopic determinations of hyphal biovolume (5, 6, 8, 15), ATP concentrations (14, 21, 23, 27), and, more recently, ergosterol concentrations (13, 14). Microscopic methods are generally thought to underestimate the quantity of fungal biomass associated with decaying leaves because of the difficulty of separating hyphae from the leaf substratum (17). A shortcoming of the ATP method is that this method is not specific for fungi, i.e., concentrations of ATP reflect total living biomass. Even so, estimates of maximum concentrations of fungal biomass associated with leaves in streams based on concentrations of ATP (see references 6 and 25) and ergosterol (13) appear to be similar. However, it is difficult to make direct comparisons between methods for determining fungal biomass, since studies have rarely determined more than one index of biomass on leaves from the same stream (e.g., see references 8 and 14).

Aquatic hyphomycete production associated with decomposing leaves includes both the growth of mycelia within leaf tissue and the formation of conidia. In these fungi, a significant proportion of production is used in the formation of conidia which are released from leaves (23). The reproductive potential of mycelia of different fungal species within leaves can be estimated by determining sporulation rates (2).

This provides an additional method with which an important fungal activity can be assessed.

Our main objective of the present study was to compare ATP and ergosterol concentrations as indicators of fungal biomass. To make comparisons without interference by other organisms, we determined the changes in concentrations of ATP and ergosterol associated with leaf material that had been inoculated with aquatic hyphomycetes in laboratory microcosms. We also compared the concentrations of both compounds associated with leaves decomposing in two streams in which microorganisms other than fungi contributed to the ATP pool. In all studies, the sporulation rates of fungi colonizing leaves were also determined to compare patterns of fungal reproductive activity with patterns of growth.

## MATERIALS AND METHODS

**Laboratory studies.** Single-spore isolates of *Anguillospora filiformis* Greathead from Alabama (AL 4) and *Flagellospora curvula* Ingold from France (CERR 30.67) were used in culture studies. Each was grown in aeration chambers (23) containing 15 tulip poplar (*Liriodendron tulipifera* L.) leaf disks (diameter, 11.4 mm) which had been sterilized by gamma irradiation (3 Mrads) and 40 ml of an inorganic salt solution containing 0.5 g of MOPS (morpholinopropane sulfonic acid) as buffer, 0.1 g of KNO<sub>3</sub>, 0.025 g of K<sub>2</sub>HPO<sub>4</sub>, 0.1 g of CaCl<sub>2</sub> · H<sub>2</sub>O, 0.01 g of MgSO<sub>4</sub>, and 1 liter of H<sub>2</sub>O (pH adjusted to 7). Solutions of Ca and Mg salts and sterile leaf disks were added after all solutions had been autoclaved. Cultures were inoculated with 3,000 conidia of the appropriate fungus and aerated with air (100 ml/min) which was filtered through cotton plugs inserted in the aeration tubes of the chambers (23). Fluid in the chambers was removed and replaced with fresh solution after the first 24 h and at 48-h intervals throughout the study by a sterile technique. At each sampling interval, rates of sporulation

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TABLE 1. Physicochemical characteristics of Big Sandy Creek and the Touyre during the studies<sup>a</sup>

Stream	Discharge (m <sup>3</sup> /s)	Temp (°C)	pH	Alkalinity (meq/liter)	NO <sub>3</sub> -N (μg/liter)	PO <sub>4</sub> -P (μg/liter)	Total dissolved P
Big Sandy Creek	0.48 (0.40–0.55)	16.2 (13–18)	8.0 (7.9–8.1)	2.48 (2.34–2.58)	260 (247–267)	80 (76–84)	
Touyre	0.34 (0.19–1.86)	6.1 (0.4–11.6)	7.7 (7.5–8.1)	0.26 (0.22–0.33)	180 (100–260)		10 (9–14)

<sup>a</sup> Values are the means, with the ranges given in parentheses.

and the cumulative total conidium production were determined by filtering known aliquots of the fluid or dilutions of the fluid, staining the conidia with 0.1% trypan blue in lactic acid, and counting the conidia in 25 fields for each filter (23). At each sampling time, two filters were counted for each of the triplicate chambers that were randomly chosen for counting. On six dates during each incubation, three chambers were sacrificed. Leaf disks from the chambers were divided into three groups for determination of dry weight remaining and of ATP and ergosterol concentrations (see below).

**Stream studies.** Leaves were placed in Big Sandy Creek, a second-order, hardwater stream in central Alabama (33°07'N, 87°20'W) and the Touyre, a third-order, softwater stream in the French Pyrenees (42°52'N, 1°45'E). The physicochemical characteristics of the two streams are listed in Table 1. Both studies were begun in October, 1990. The study at Big Sandy Creek used tulip poplar leaves (*Liriodendron tulipifera* L.) which had been naturally shed in the autumn of 1990 and dried at room temperature for 1 to 2 months. Each leaf was placed in a separate 1.2-mm-mesh bag and suspended from strips of wood (2 by 4 cm by 2.4 m) which were held in the current off the bottom by concrete blocks (23). The study at the Touyre used freshly shed alder leaves (*Alnus glutinosa* (L.) Gaertn.), of which 13 g (wet weight), corresponding to ca. 5 g (dry weight), was placed in 9-mm-mesh bags, tied to bricks, and introduced into the stream (9).

**Sporulation rates.** To determine sporulation rates from leaf litter in Big Sandy Creek, 15 leaf disks (diameter, 11.4 mm) from each triplicate litter bag were placed in aeration chambers with 40 ml of filtered stream water and aerated for 24 h; from the Touyre, one leaf from each triplicate pack was aerated in 150 ml of filtered stream water for 48 h. The resulting suspensions of conidia were filtered through membrane filters and counted as described above.

**Chemical analyses.** ATP was extracted by homogenization of leaf disks in sulfuric-oxalic acids and quantified by using a Turner luminometer to measure luciferin-luciferase light emission (26). Values were corrected on the basis of internal standards incorporated with each part of the study. Ergosterol was extracted by refluxing for 30 min in alcoholic base and was quantified by measuring the  $A_{282}$  after separation with high-performance liquid chromatography (10).

**Conversion factors.** To determine mycelial dry mass from ATP and ergosterol concentrations, conversion factors were determined from aquatic hyphomycetes grown in a mineral salt solution amended with glucose. Mycelia from replicate flasks were harvested during late growth phase and were analyzed for dry weight and either ATP (23) or ergosterol (11).

## RESULTS

The increases in concentrations of ATP associated with leaf material colonized by either *A. filiformis* (Fig. 1A) or *F.*

*curvula* (Fig. 2A) were similar to the patterns of increase in ergosterol concentrations during the growth phase of each fungus. Differences between the patterns of ATP and ergosterol concentrations occurred as the fungi entered stationary phase. Overall, ATP concentrations were significantly correlated with ergosterol concentrations for both fungi (Table 2).

Sporulation data from fungi grown in the laboratory are presented both as sporulation rate (Fig. 1B and 2B [rate]) and cumulative output (Fig. 1B and 2B [total]) of conidia. Cumulative output of conidia presents sporulation data in a format similar to that for ATP and ergosterol data, i.e., the data are measurements of concentrations or amounts rather than rates. For both fungi, increases in cumulative output of conidia lagged behind mycelial growth (increases in ATP and ergosterol concentrations) by 2 to 4 days. The greatest rates of sporulation by both fungi occurred at the end of the growth phase (19 days for *A. filiformis* [Fig. 1B] and 17 days for *F. curvula* [Fig. 2B]). ATP and ergosterol concentrations were significantly correlated with the cumulative output of conidia by each fungus and with the sporulation rates of *A. filiformis* (Table 2). However, neither of the biomass indices was significantly correlated with the sporulation rates of *F. curvula*.

Conversion factors for the calculation of fungal biomass

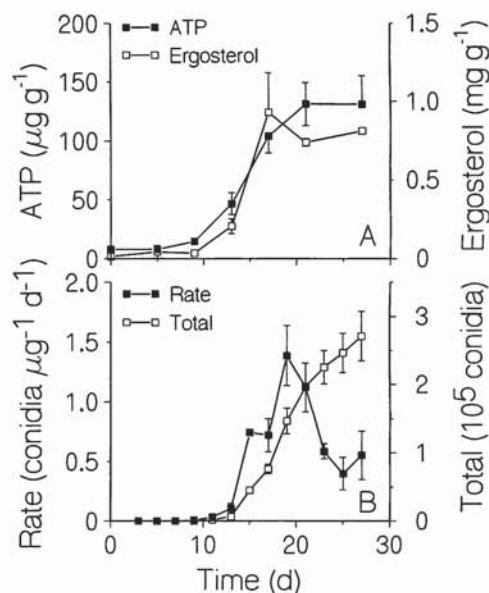


FIG. 1. Changes in tulip poplar leaf disks colonized by *A. filiformis* in laboratory microcosms. (A) ATP and ergosterol concentrations associated with leaf disks; (B) sporulation rates and total output of conidia from the fungus growing on leaf disks. Symbols at each sampling date represent means  $\pm$  1 standard error of the mean (SEM). d, day.

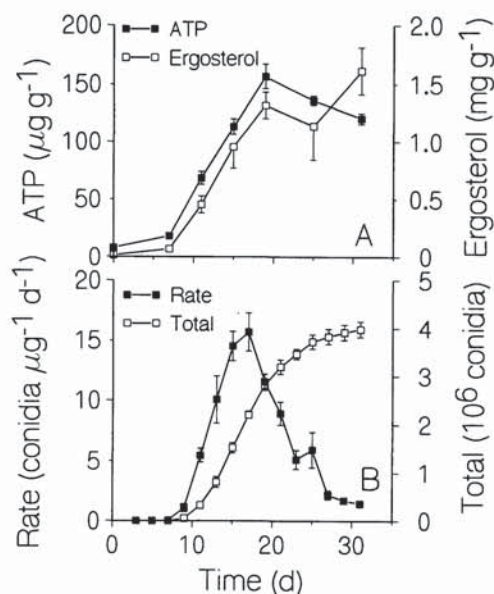


FIG. 2. Changes in tulip poplar leaf disks colonized by *F. curvula* in laboratory microcosms. (A) ATP and ergosterol concentrations associated with leaf disks; (B) sporulation rates and total output of conidia from the fungus growing on leaf disks. Symbols at each sampling date represent means  $\pm$  1 SEM. d, day.

from either ATP or ergosterol concentrations were determined for both fungal species. Mean ATP concentrations were  $1.80 \text{ mg g}^{-1}$  for *A. filiformis* (23) and  $1.63 \text{ mg g}^{-1}$  (standard deviation, 0.18,  $n = 8$ ) for *F. curvula*. Mean ergosterol concentrations were  $10.9 \text{ mg g}^{-1}$  (standard deviation, 0.3,  $n = 3$ ) for *A. filiformis* and  $10.0 \text{ mg g}^{-1}$  for *F. curvula* (11). Estimates of biomass associated with leaf material calculated from ergosterol concentrations averaged one to two times those calculated from ATP for both fungi (Table 3). The maximum levels of fungal biomass associated with leaves colonized by *A. filiformis* ranged from 7% (based on ATP) to 8.5% (based on ergosterol) of the dry weight of the leaf plus fungus. The maximum levels of *F. curvula* biomass under these conditions ranged from 9.6% (based on ATP) to 16% (based on ergosterol) of the dry weight of the leaf plus fungus.

Changes in ATP concentrations associated with tulip poplar leaves decomposing in Big Sandy Creek exhibited

patterns which were similar to those for changes in ergosterol concentrations during the initial colonization period (Fig. 3A). The maximum concentrations of both compounds occurred at 14 days, which were followed by declines. ATP and ergosterol were positively correlated in Big Sandy Creek (Table 2). The maximum rates of sporulation by aquatic hyphomycetes growing on leaves in Big Sandy Creek also occurred at 14 days (Fig. 3B) and were significantly correlated with both ATP and ergosterol concentrations (Table 2).

In the Touyre, ATP and ergosterol concentrations were negatively correlated (Table 2). Here, the ATP contents of the fresh alder leaves were high initially, declined through 21 days, and then remained relatively constant (Fig. 4A). The ergosterol concentrations of leaves in the Touyre increased gradually through 28 days and then remained relatively constant throughout the remainder of the study. The greatest rates of sporulation by aquatic hyphomycetes growing on leaves in the Touyre occurred at 28 days (Fig. 4B). In the Touyre, neither ATP nor ergosterol concentrations were significantly correlated with sporulation rates.

The conversion factors that we used to calculate fungal biomass associated with leaves colonized in streams were based on averages from several fungi. These were  $1.75 \text{ mg g}^{-1}$  for ATP (23 and the present study) and  $5.5 \text{ mg g}^{-1}$  for ergosterol (11). With these conversion factors, the fungal biomass based on ergosterol concentrations was one to three times that determined from ATP concentrations in both streams (Table 3), even though ATP included the biomass of other microorganisms. In Big Sandy Creek, maximum fungal biomass occurred at 14 days and reached 9% (based on ATP) to 16% (based on ergosterol) of the weight remaining. In the Touyre, maximum fungal biomass ranged from 5% (based on ATP at 28 days) to 13% (based on ergosterol at 56 days) of the weight remaining.

## DISCUSSION

The good correspondence between the patterns of change in ATP and ergosterol concentrations of leaves colonized in the laboratory (Fig. 1 and 2) and in Big Sandy Creek (Fig. 3) suggests that both compounds reflect changes in the biomass of fungi colonizing leaves in these systems. Golladay and Sinsabaugh (14) also found a positive correlation between ATP and ergosterol concentrations of leaves decomposing in a stream. Although ATP, unlike ergosterol, is not specific for fungi, it is not surprising that ATP and ergosterol concentrations were highly correlated in these systems, because the microbial assemblages colonizing leaf litter in streams are generally dominated by fungi. For example, Findlay and

TABLE 2. Correlation coefficients

Type of leaf colonization	Correlation coefficient				
	ATP-ergosterol	ATP-sporulation rate	ATP-cumulative output	Ergosterol-sporulation rate	Ergosterol-cumulative output
Laboratory colonization (n)					
<i>A. filiformis</i> (7)	0.955 <sup>a</sup>	0.918 <sup>a</sup>	0.906 <sup>a</sup>	0.885 <sup>b</sup>	0.796 <sup>b</sup>
<i>F. curvula</i> (7)	0.928 <sup>a</sup>	0.681 <sup>c</sup>	0.861 <sup>b</sup>	0.456 <sup>c</sup>	0.941 <sup>a</sup>
Stream colonization					
Big Sandy Creek (7)	0.847 <sup>b</sup>	0.795 <sup>b</sup>		0.851 <sup>b</sup>	
Touyre (8)	-0.775 <sup>b</sup>	-0.634 <sup>c</sup>		0.564 <sup>c</sup>	

<sup>a</sup>  $P < 0.005$ .

<sup>b</sup>  $P < 0.05$ .

<sup>c</sup>  $P > 0.05$ .

TABLE 3. Ratios of estimates of fungal biomass associated with leaves<sup>a</sup>

Fungal species or stream	Mean ergosterol biomass: ATP biomass ratio (range)	Time interval considered (days)
<i>A. filiformis</i>	1.0 (0.7–1.5)	13–27
<i>F. curvula</i>	1.5 (1.1–2.2)	11–31
Big Sandy Creek	1.5 (0.6–2.4)	7–51
Touyre	2.4 (2.2–2.7)	28–56

<sup>a</sup> Biomass was calculated from ergosterol and ATP concentrations in laboratory microcosms and field studies.

Arsuffi (8) used conservative microscopic methods (17) and estimated that fungi account for 84 to 96% of the total microbial biomass associated with the leaves submerged in their study stream. Thus, both ATP and ergosterol appear to be useful indicators of the amount of living fungal biomass associated with decomposing leaf litter in streams.

On substrata other than leaf litter, biomass calculated from ATP concentrations does not necessarily reflect fungal biomass. For example, ergosterol and ATP concentrations are not significantly correlated in epixylic or epilithic communities in streams (14, 22). High concentrations of chlorophyll *a* associated with wood and glass slides (14, 22) suggest that ATP concentrations reflect the presence of considerable algal biomass on these substrata.

In contrast to the results with leaves in the laboratory and in Big Sandy Creek, concentrations of ATP and ergosterol associated with freshly shed alder leaves placed in the Touyre were negatively correlated with one another (Fig. 4A; Table 2). Low ergosterol concentrations associated with leaves from the Touyre during the first 1 to 2 weeks of submersion indicate that fungal biomass was low during this period (Fig. 4A). The pattern observed for these leaves

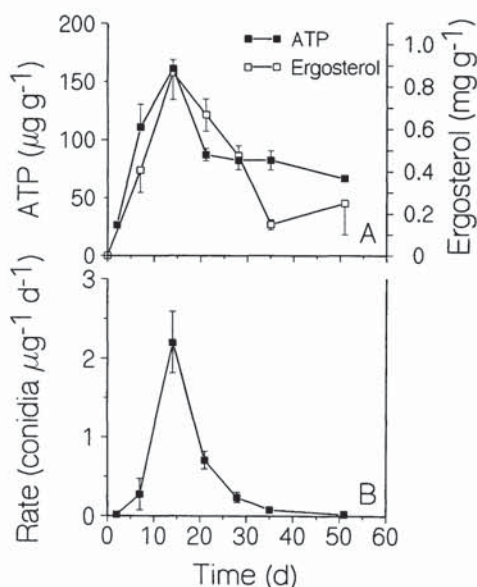


FIG. 3. Changes in tulip poplar leaves in Big Sandy Creek. (A) ATP and ergosterol concentrations associated with leaves; (B) sporulation rates of aquatic hyphomycetes growing on leaves. Symbols at each sampling date represent means  $\pm$  1 SEM. d, day.

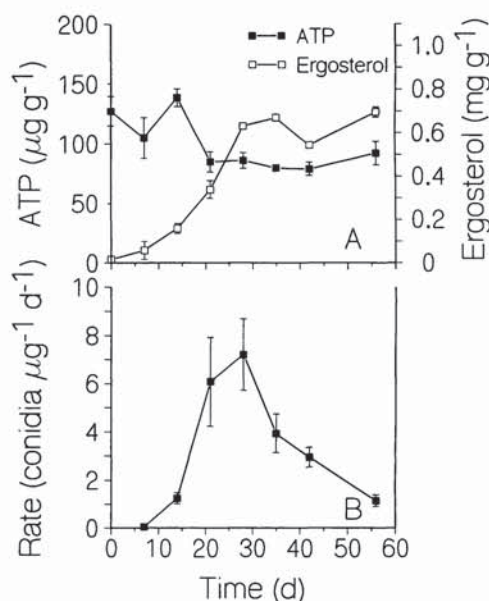


FIG. 4. Changes in alder leaves in the Touyre. (A) ATP and ergosterol concentrations associated with leaves; (B) sporulation rates of aquatic hyphomycetes growing on leaves. Symbols at each sampling date represent means  $\pm$  1 SEM. d, day.

suggests that leaf-derived ATP persisted during the first 2 to 3 weeks of exposure in the stream. Freshly shed leaves lose soluble components gradually when submerged in streams (4, 9, 12), whereas dried leaves leach soluble components rapidly (20). These observations suggest that cellular integrity and metabolic activity in fresh leaves are maintained well beyond abscission. Therefore, the high ATP concentrations during the first 2 to 3 weeks in the Touyre appear to be due to the leaf tissue itself rather than to associated fungi. It seems unlikely that other microorganisms, e.g., bacteria, protozoa, or algae, could have contributed this amount to the ATP pool in such a short time.

In the laboratory studies, the cumulative output of conidia was significantly correlated with ATP and ergosterol concentrations for both fungi, but the sporulation rates were not significantly correlated with these biomass indices for *F. curvula*. The cumulative output of conidia is calculated by integrating sporulation rates through time. This can be readily done in the laboratory, in which the temperature is constant and all conidia accumulate in the chambers between sampling dates. However, in stream studies, the time between sampling is longer than in laboratory studies, and sporulation rates may vary between sample dates in response to changes in temperature and other environmental variables. Consequently, estimations of cumulative conidium production in field studies would be much less accurate than they are in laboratory studies. In both field studies, we compared only the relationships between sporulation rates and concentrations of the biomass indicators ATP and ergosterol. These parameters exhibited significant, positive correlations in Big Sandy Creek but not in the Touyre. It is difficult to determine whether this represents another difference between air-dried and freshly shed leaves or is due to the differences in the fungal assemblages in these streams. However, it is interesting to note that, in line with the second explanation, the sporulation rate was not correlated with

either of the biomass indices in the laboratory study with *F. curvula*, one of the dominant fungi on leaves in the Touyre.

In both laboratory and field studies, patterns of changes in ATP and ergosterol concentrations and sporulation rates were similar during the active growth phase and exhibited greater differences during the stationary phase. Aquatic hyphomycetes sporulated during periods of growth and sporulation corresponded rather closely with increases in biomass. These results are similar to results from other streams (2, 21, 23) and suggest that all three parameters measured in the present study give similar types of information when aquatic hyphomycete mycelia are active. Only when the mycelium becomes senescent, stressed, or inactive does the information provided by these methods become more difficult to interpret.

In the present study, estimates of fungal biomass based on ergosterol concentrations tended to be one to three times those based on ATP concentrations (Table 3). Application of the same conversion factors for ATP and ergosterol to the maximum concentrations reported by Golladay and Sinsabaugh (14) also results in higher (4.4 times) biomass for ergosterol than for ATP. The most likely explanation for these inequalities is that the conversion factors that we used (derived from aquatic hyphomycetes grown in liquid culture) do not accurately reflect the relationship between concentrations of one or both of the biomass indices and the fungal dry weight when these organisms are growing inside the leaf matrix (17, 19). Cellular concentrations of ergosterol in fungi depend on a variety of factors, including growth conditions and taxonomic affiliation (11, 17, 19). In particular, hyphae of the same fungus may vary considerably, depending on whether they are grown in liquid or solid media and whether they are grown on leaf extracts or on standard laboratory media (11, 17). Significant variation may even occur among strains of the same species (11).

Less information on the variability of ATP concentrations in aquatic hyphomycetes is available. However, in other microorganisms, ATP concentrations are known to vary depending on the metabolic activity and growth stage (1, 16). Since it is a functional cell constituent with a very rapid turnover, ATP has even been proposed as an indicator of metabolic stress (7), i.e., as a descriptor of physiological state rather than of biomass. If fungi experience stress during sampling or sample processing, they may respond with decreasing ATP concentrations, which would lead to an underestimation of fungal biomass. However, this explanation is unlikely to apply for the fungi grown on leaves in the microcosms, because the same phenomenon would probably have occurred when the conversion factors were established. As a structural component of fungal membranes, ergosterol is probably less affected by stress even though the molecule is degraded rather quickly upon cell death (18).

We conclude that both ATP and ergosterol provide similar information concerning the dynamics of fungal biomass associated with decomposing leaves in streams. In situations in which other organisms make significant contributions to the pool of ATP (e.g., freshly shed leaves and/or algae), measurement of ergosterol concentrations gives a more accurate picture of the patterns of change in fungal mass. Conversely, our estimates of the amounts of fungal biomass derived from the two chemical indices differed from one another. Amounts of fungal biomass calculated from ergosterol concentrations were generally higher than those derived from ATP concentrations. It is encouraging that the differences were relatively small; however, these results emphasize the difficulty in determining conversion factors

which accurately reflect the contents of such compounds in mycelia growing in nature.

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