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Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi

Ergosterol has recently been used as a biomass indicator to compare the growth of different arbuscular mycorrhizal (AM) fungi (Hart & Reader, 2002a,b). Here, we show that ergosterol is not a suitable biochemical marker for estimating the biomass of AM fungi and that the comparison of biomass between different fungal taxa is very difficult using any kind of currently available biochemical marker.

Because they are usually degraded rapidly after cell death and because membrane area is assumed to be well correlated with the biovolume of microbial cells (Tunlid & White, 1992), membrane compounds, such as sterols, are attractive biomass indicators of microorganisms in environmental samples. Furthermore, sterols seem to represent a rather constant part of the fungal biomass, constituting somewhere between 5 and 15 mg g\(^{-1}\) in most fungal groups (Weete & Gandhi, 1996). In particular, ergosterol is specific to the fungal kingdom (Weete & Gandhi, 1996) and occurs mainly as a membrane constituent. Ergosterol has been used to indicate the fungal biomass in soil (Grant & West, 1986; Frostegård & Bååth, 1996), pathogenic fungi in roots (Bindler et al., 1988), fungi in cereal grains (Seitz et al., 1972), saprophytic fungi in decaying plant material (Newell et al., 1988) and ectomycorrhizal fungi in roots (Salmanowicz & Nylund, 1988; Wallander et al., 1997) and soil (Ek et al., 1994; Ekblad et al., 1995).

The occurrence of ergosterol is generally restricted to the more advanced fungal taxa, while the more primitive taxa contain other sterols (Weete & Gandhi, 1996). Thus, it is the dominating sterol in ascomycetes and basidiomycetes. By contrast, the picture is rather more complex within the phylum Zygomycota where members of Mucorales contain ergosterol, while Mortierella contain desmosterol, but no ergosterol (Weete & Gandhi, 1999). In a similar way, most members of the newly identified phylum Glomeromycota (Schüssler et al., 2001), fungal obligate symbionts forming arbuscular mycorrhizas (AM), seem to contain sterols other than ergosterol. No ergosterol was detected in several studies in which gas chromatography-mass spectrometry (GC-MS) analysis was carried out on spores or extraradical mycelium of either Glomus or Acaulospora species (Beilby & Kidby, 1980; Beilby, 1980; Nordby et al., 1981; Grandmougin-Ferjani et al., 1999; Fontaine et al., 2001) or mature spores of Gigaspora margarita (Grandmougin-Ferjani et al., 1999). However, Frey et al. (1992, 1994) identified ergosterol in roots colonised by Glomus intraradices using GC-MS, but not in noncolonised roots, and they proposed the ergosterol content in extraradical hyphae of this fungus to be 0.063 mg per g mycelium. More recently, Fujiyoshi et al. (2000) found that the mycelium collected around roots colonised by Gigaspora margarita had 0.63 mg ergosterol per g of mycelium. Nevertheless, neither of the former two studies was carried out under in vitro conditions, and thus ergosterol from contaminating fungi could hardly be avoided. The slightest contamination may have a significant effect on the results because of the high ergosterol content in many saprophytic fungi.

Despite the fact that ergosterol has been shown to be absent in AM fungi on several occasions, high performance
liquid chromatography (HPLC) estimation of ergosterol was recently used as a means of estimating and comparing the fungal biomass of various AM fungi in soil and roots (Hart & Reader, 2002a,b). In the same studies, the ergosterol content of the AM fungal inocula was used as a means of equalising the amount of inoculum added. In order to ascertain whether ergosterol can be used to estimate AM fungal biomass at all, we investigated ergosterol content in monoxenically (*in vitro*) grown AM fungi (Petri dish systems with carrot root cultures) where no contaminating fungi could affect the results.

We collected the extraradical mycelium of *G. intraradices* developing in liquid medium of monoxenic cultures (Olsson et al., 2002) and *Gi. margarita* in solid medium (Bago et al., 2002) as well as colonised and noncolonised roots. The ergosterol contents of both extraradical mycelium and colonised roots were estimated by HPLC separation and the specific detection of ergosterol using a UV detector (Nylund & Wallander, 1992), which is a commonly used method for ergosterol determination as a fungal biomass indicator. Other sterols are retained in the purified sample but only ergosterol is detectable at 280 nm because of a conjugated pair of double bonds (Nylund & Wallander, 1992). A newly developed highly specific method in which ergosterol is analysed using tandem mass spectrometry (GC-MS-MS; Larsson & Saraf, 1997) was also used. Irrespective of the method, we found no detectable ergosterol in *G. intraradices* or *Gi. margarita* (Table 1), which is in accordance with earlier studies. The fatty acid 16 : 1 ω5 has been used as a biomarker for AM fungi in many studies (Olsson et al., 1995; Olsson, 1999), but even with this method it is difficult to make comparisons between species because the variation between species and between genera may be considerable (Graham et al., 1995). In order to use any biochemical marker for estimating AM fungal biomass a conversion factor for each species must first be obtained, ideally from monoxenic cultures. The large variation that can be found in any biochemical marker compound is furthermore exemplified by the variation we

<table>
<thead>
<tr>
<th>Biological materials</th>
<th>Ergosterol (mg g⁻¹)</th>
<th>HPLC</th>
<th>GC-MS-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomalean fungi (extraradical mycelium)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Glomus intraradices</em></td>
<td>&lt; 0.025</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td><em>Gigaspora margarita</em></td>
<td>&lt; 0.16</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Saprophytic zygomycetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rhizopus arhizus</em></td>
<td>2.9</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td><em>Zygorhynchus heterogamus</em></td>
<td>3.1</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Ascomycetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Penicillium roqueforti</em> (mainly conidia)</td>
<td>0.07</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td><em>Cenococcum geotropis</em></td>
<td>4.2</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Ectomycorrhizal basidiomycete</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Paxillus involutus</em></td>
<td>8.5</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Carrot roots</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonmycorrhizal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Glomus intraradices</em></td>
<td>&lt; 0.006</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td><em>Gigaspora margarita</em></td>
<td>&lt; 0.010</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Seven different mycelia of *G. intraradices* was analysed and one of *Gi. margarita*. Ergosterol was measured either with high performance liquid chromatography (HPLC) or with tandem mass spectrometry (GC-MS-MS).

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Ergosterol (mg g⁻¹)</th>
<th>PLFA 18 : 2ω6,9 (µmol g⁻¹)</th>
<th>Ratio PLFA 18 : 2ω6,9/ergosterol (µmol mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Paxillus involutus</em></td>
<td>4.5 ± 1.6</td>
<td>2.1 ± 0.5</td>
<td>0.45</td>
</tr>
<tr>
<td><em>Suillus bovinus</em></td>
<td>5.4 ± 3.5</td>
<td>24.3 ± 4.4</td>
<td>4.5</td>
</tr>
<tr>
<td><em>Suillus variegatus</em></td>
<td>1.8 ± 0.7</td>
<td>21.5 ± 2.2</td>
<td>12</td>
</tr>
</tbody>
</table>

The PLFA content was measured with GC (Olsson et al., 1995) and ergosterol with HPLC.
found in the content of the phospholipid fatty acid (PLFA) 18:0,6,9 between *Sulphur* spp. and *Pseudolus* *involutus*, while both fungi contained similar amounts of ergosterol (Table 2).

We conclude that: ergosterol cannot be used as biomass indicator for glomalean fungi; and regardless of which biochemical marker is used, it is very difficult to make comparisons of biomass between different species because there are always taxonomic-based differences in content of any signature compound. Ideally, a specific conversion factor would have to be developed for each taxa when the aim is to compare the growth of different species.

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References


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