



LUND UNIVERSITY

Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi

Olsson, Pål Axel; Larsson, L; Bago, B; Wallander, Håkan; van Aarle, Ingrid

Published in:
New Phytologist

DOI:
[10.1046/j.1469-8137.2003.00810.x](https://doi.org/10.1046/j.1469-8137.2003.00810.x)

2003

[Link to publication](#)

Citation for published version (APA):

Olsson, P. A., Larsson, L., Bago, B., Wallander, H., & van Aarle, I. (2003). Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi. *New Phytologist*, 159(1), 7-10. <https://doi.org/10.1046/j.1469-8137.2003.00810.x>

Total number of authors:
5

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

- Ronning CM, Stegalkina SS, Ascenzi RA, Bougri O, Hart AL, Utterbach TR, Vanaken SE, Riedmüller SB, White JA, Cho J, Perteza GM, Lee Y, Karamycheva S, Sultana R, Tsai J, Quackenbush J, Griffiths HM, Restrepo S, Smart CD, Fry WE, van der Hoeven R, Tanksley S, Zhang P, Jin H, Yamamoto ML, Baker BJ, Buell RC. 2003. Comparative analyses of potato expressed sequence tag libraries. *Plant Physiology* 131: 419–429.
- Skinner W, Keon J, Hargreaves J. 2001. Gene information for fungal plant pathogens from expressed sequences. *Current Opinions in Microbiology* 4: 381–386.
- Tunlid A. 2003. Exploring plant–microbe interactions using DNA microarrays. *New Phytologist* 158: 235–238.
- Voiblet C, Duplessis S, Encelot N, Martin F. 2001. Identification of symbiosis-regulated genes in *Eucalyptus globulus*–*Pisolithus tinctorius* ectomycorrhiza by differential hybridization of arrayed cDNAs. *Plant Journal* 25: 181–191.

Key words: transcriptome, *Pisolithus*, *Laccaria*, ectomycorrhiza, genomics.

Letters

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

Biological materials	Ergosterol (mg g ⁻¹)	
	HPLC	GC-MS-MS
Glomalean fungi (extraradical mycelium)		
<i>Glomus intraradices</i>	< 0.025	< 0.001
<i>Gigaspora margarita</i>	< 0.16	< 0.001
Saprophytic zygomycetes		
<i>Rhizopus arrhizus</i>	2.9	4.8
<i>Zygorrhynchus heterogamus</i>	3.1	3.9
Ascomycetes		
<i>Penicillium roqueforti</i> (mainly conidia)	0.07	0.41
<i>Cenococcum geoforme</i>	4.2	3.5
Ectomycorrhizal basidiomycete		
<i>Paxillus involutus</i>	8.5	5.9
Carrot roots		
Nonmycorrhizal	< 0.006	< 0.0001
<i>Glomus intraradices</i>	< 0.002	< 0.0001
<i>Gigaspora margarita</i>	< 0.010	< 0.0001

Table 1 Ergosterol content of fungal mycelia or monoxenic carrot root colonised with AM fungi or nonmycorrhizal

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 2 The content of the PLFA 18 : 2ω6,9 and ergosterol (± SD) in mycelia of *Suillus* spp. ($n = 8$, including two isolates of each species) and *Paxillus involutus* ($n = 12$, including three isolates) growing in pure culture on agar, and the ratio of PLFA 18 : 2ω6,9 to ergosterol

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

The PLFA content was measured with GC (Olsson *et al.*, 1995) and ergosterol with HPLC.

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 : 1ω5 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

found in the content of the phospholipid fatty acid (PLFA) 18 : 206,9 between *Suillus* spp. and *Paxillus 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.

Acknowledgements

We thank Christina Pehrsson and Custodia Cano for technical assistance, and Erland Bååth for valuable suggestions.

**Pål Axel Olsson^{1,*}, Lennart Larsson², Bert Bago³,
Håkan Wallander¹ and Ingrid M. van Aarle¹**

¹Department of Microbial Ecology, Ecology Building,
Lund University, SE-223 62 Lund, Sweden;

²Department of Medical Microbiology, Division of
Bacteriology, Lund University, Sölvegatan 23, SE 223 62
Lund, Sweden; ³Centro de Investigaciones sobre
Desertification (CSIC/UV/GV), Valencia, Spain
(*Author for correspondence tel +46 46 2229614;
fax +46 46 2224158;
email Pal_Axel.Olsson@mbioekol.lu.se)

References

- Bago B, Zipfel W, Williams RC, Jun J, Arreola R, Pfeffer PE, Lammers PJ, Shachar-Hill Y. 2002. Translocation and utilization of fungal storage lipid in the arbuscular mycorrhizal symbiosis. *Plant Physiology* 128: 108–124.
- Beilby JP. 1980. Fatty acid and sterol composition of ungerminated spores of the vesicular-arbuscular mycorrhizal fungus, *Acaulospora laevis*. *Lipids* 15: 949–952.
- Beilby JP, Kidby DK. 1980. Sterol composition of ungerminated and germinated spores of the vesicular-arbuscular mycorrhizal fungus, *Glomus caledonius*. *Lipids* 15: 375–378.
- Bindler GN, Piadé JJ, Schulthess D. 1988. Evaluation of selected steroids as chemical markers of past or presently occurring fungal infections on tobacco. *Beiträge Zur Tabakforschung International* 14: 127–134.
- Ek H, Sjögren M, Arnebrant K, Söderström B. 1994. Extramatricel mycelial growth, biomass allocation and nitrogen uptake in ectomycorrhizal systems in response to collembolan grazing. *Applied Soil Ecology* 1: 155–169.
- Ekblad A, Wallander H, Carlsson R, Huss-Danell K. 1995. Fungal biomass in roots and extramatricel mycelium in relation to macronutrients and plant biomass of ectomycorrhizal *Pinus sylvestris* and *Abies incana*. *New Phytologist* 131: 443–451.
- Fontaine J, Grandmougin-Ferjani A, Hartmann M-A, Sancholle M. 2001. Sterol biosynthesis by the arbuscular mycorrhizal fungus *Glomus intraradices*. *Lipids* 36: 1357–1363.
- Frey B, Buser HR, Schüepp H. 1992. Identification of ergosterol in vesicular-arbuscular mycorrhizae. *Biology and Fertility of Soils* 13: 229–234.
- Frey B, Vilarino A, Schüepp H, Arines J. 1994. Chitin and ergosterol content of extraradical and intraradical mycelium of the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices*. *Soil Biology and Biochemistry* 26: 711–717.
- Frostegård Å, Bååth E. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils* 22: 59–65.
- Fujiyoshi M, Nakatsubo T, Ogura S, Horikoshi T. 2000. Estimation of mycelial biomass of arbuscular mycorrhizal fungi associated with the annual legume *Kummerowia striata* by ergosterol analysis. *Ecological Research* 15: 121–131.
- Graham JH, Hodge NC, Morton JB. 1995. Fatty acid methyl ester profiles for characterization of glomalean fungi and their endomycorrhizae. *Applied and Environmental Microbiology* 61: 58–64.
- Grandmougin-Ferjani A, Dalpé Y, Hartmann M-A, Laurelle F, Sancholle M. 1999. Sterol distribution in arbuscular mycorrhizal fungi. *Phytochemistry* 50: 1027–1031.
- Grant WD, West AW. 1986. Measurement of ergosterol, diaminopimelic acid and glucosamine in soil: evaluation as indicators of microbial biomass. *Journal of Microbiological Methods* 6: 47–53.
- Hart MM, Reader RJ. 2002a. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist* 153: 335–344.
- Hart MM, Reader RJ. 2002b. Host plant benefit from association with arbuscular mycorrhizal fungi: variation due to differences in size of mycelium. *Biology and Fertility of Soils* 36: 357–366.
- Larsson L, Saraf A. 1997. Use of gas chromatography-ion trap tandem mass spectrometry for the detection and characterization of microorganisms in complex samples. *Molecular Biotechnology* 7: 279–287.
- Newell SY, Arsuffi TL, Fallon RD. 1988. Fundamental procedures for determining ergosterol content of decaying plant material by liquid chromatography. *Applied and Environmental Microbiology* 54: 1876–1879.
- Nordby HE, Nemeč S, Nagy S. 1981. Fatty acid and sterols associated with *Citrus* root mycorrhizae. *Journal of Agricultural Food Chemistry* 29: 396–401.
- Nylund J-E, Wallander H. 1992. Ergosterol analysis as a means of quantifying mycorrhizal biomass. In: Norris JR, Read DJ, Varma AK, eds. *Methods in microbiology*, Vol. 24. London, UK: Academic Press, 77–88.
- Olsson PA. 1999. Signature fatty acids provide tools for determination of distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiology Ecology* 29: 303–310.
- Olsson PA, Bååth E, Jakobsen I, Söderström B. 1995. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. *Mycological Research* 99: 623–629.
- Olsson PA, Van Aarle IM, Allaway WG, Ashford AE, Rouhier H. 2002. Phosphorus effects on metabolic processes in monoxenic arbuscular mycorrhiza cultures. *Plant Physiology* 130: 1162–1171.

- Salmanowicz B, Nylund J-E. 1988. High performance liquid chromatography determination of ergosterol as a measure of ectomycorrhizae infection in Scots pine. *European Journal of Forest Pathology* **18**: 291–298.
- Schüssler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* **105**: 1413–1421.
- Seitz LM, Mohr HF, Burroughs R, Sauer DB. 1972. Ergosterol as an indicator of fungal invasion in grains. *Cereal Chemistry* **54**: 1207–1217.
- Tunlid A, White DC. 1992. *Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil*. *Soil biochemistry, Vol. 7*. New York, NY, USA: Dekker, 229–262.
- Wallander H, Massicotte HB, Nylund J-E. 1997. Seasonal variation in protein, ergosterol and chitin in five morphotypes of *Pinus sylvestris* L. ectomycorrhizae in a mature Swedish forest. *Soil Biology and Biochemistry* **29**: 45–53.
- Weete JD, Gandhi SR. 1996. Biochemistry and molecular biology of fungal sterols. In: Brambl R, Marzluf G, eds. *The mycota III biochemistry and molecular biology*. Berlin, Germany: Springer, 421–438.
- Weete JD, Gandhi SR. 1999. Sterols and fatty acids of the Mortierellaceae: taxonomic implications. *Mycologia* **91**: 642–649.

Key words: ergosterol, fatty acids, biomass estimation, mycorrhiza.