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Short communication

Ergosterol content in ericaceous hair roots correlates with dark septate endophytes but not with ericoid mycorrhizal colonization

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Abstract

The relationship between ergosterol content in ericaceous hair roots and ericoid mycorrhizal (ErM) colonization versus dark septate endophytic (DSE) hyphal colonization was examined in a dwarf shrub-dominated subarctic mire in Northern Sweden. Ergosterol content in hair roots did not correlate with ErM colonization in corresponding root samples. However, a significant positive relationship was found between hair root DSE hyphal colonization and ergosterol content. This is the first study to demonstrate that ergosterol cannot be used as a colonization indicator for ErM in hair roots growing under natural conditions. It also suggests the possibility of using ergosterol as an estimate of DSE hyphal colonization in ericaceous dwarf shrubs. This study has implications for the interpretation of results in field studies where ergosterol was used as a sole proxy for ErM colonization.

Keywords: Ergosterol; Ericoid mycorrhizal colonization; Dark septate endophytes; Visual determination

1. Introduction

Arctic and boreal regions are characterized by the dominance of ericaceous dwarf shrubs and raw humus soils with a slow turnover rate of organic matter. Essential nutrients, such as N and P are almost exclusively bound in organic compounds (Specht, 1979; Read and Kerley, 1995). There is an increasing amount of evidence that ericoid mycorrhizal (ErM) fungi produce enzymes to release amino acids and amino sugars from detrital material, which can then become available for plant uptake (Read, 1991; Näsholm et al., 1998; Read and Perez-Moreno, 2003).

Dark septate endophytic (DSE) fungi were recorded in most ericaceous dwarf shrubs also colonized with ErM fungi (Jumpponen and Trappe, 1998), and they are the most abundant root colonizers in arctic–alpine primary successions (Cazares et al., 2005). The DSE fungi are a poorly defined group of ascomycetes, and their ecological functions are little understood. Like mycorrhizal associations, DSE associations can either stimulate or reduce host plant growth (Jumpponen, 2001).

Recent knowledge on the broad ecological significance of ErM fungi and the frequent presence of DSE in heath ecosystems sharpens the need to obtain a satisfactory quantification method for these two fungal groups. Visual determination is time consuming and to some extent, subjective. Ergosterol analysis is one of the most commonly used methods for estimating fungal biomass (Nylund and Wallander, 1992). However, it has never been investigated if ergosterol can be used as a biomarker for ErM and DSE colonization. Despite this fact, ergosterol has already been used as a biomarker for ErM colonization, which reflects the need of a proper biomarker for this type of mycorrhizae (Caporn et al., 1995; Genney et al., 2000; Olsrud et al., 2004). While ergosterol has not been investigated in DSE fungi, the ergosterol content in ErM fungi was determined by Padgett and Posey (1993) as similar to the amount generally found in ascomycetes and basidiomycetes (Weete and Gandhi, 1996).

The aims of this study were (1) to investigate whether ergosterol content in hair roots could be used to estimate

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ErM colonization and (2) to determine how DSE hyphal colonization is related to ergosterol content in hair roots.

The study was performed in August 2003 in a subarctic mire near Abisko in northern Sweden (68°21′N, 19°00′E). The study site is dominated by dwarf shrubs, in particular *Empetrum hermaphroditum, Andromeda polifolia, Vaccinium uliginosum* and *Vaccinium vitis-idaea*. Further details on the study site are given by Olsrud and Christensen (2004).

A total of 15 plots, 22 × 22 cm each, were positioned at least 2 m apart within a study area covering 780 m^2. A soil core, 10 cm deep and 3.8 cm in diameter, was taken from the center of each plot on 12 August. The soil cores were transported on ice to the laboratory where they were kept in a cooling room at 2 °C. After removing the uppermost 1 cm, which consisted mostly of cryptogam biomass, the next 8 cm of the core were sorted for live hair roots (Aerts et al., 1989) on ice over 1–1.5 h in the laboratory. A representative sample of 'hair roots' was found to have the following root diameter (d) composition: 46% $d < 100$ μm, 40% $100 < d < 200$ μm, 13% $200 < d < 400$ μm and 1% $d > 400$ μm. The roots were freeze dried within 48 h after field sampling. The root samples were placed in air-tight bags together with silica gel in order to prevent any reabsorption of water and stored at 2–8 °C until further analyses during spring 2006.

A subsample of hair roots were ground, extracted and analyzed for ergosterol by high-performance liquid chromatography (HPLC) (HPLC (model L2130 VWR-Hitachi, Stockholm, Sweden), UV detector (model L2400; VWR Hitachi; Stockholm, Sweden) and a Chromolith 100–4.6 mm C18 reverse-phase column (Merck, Darmstadt, Germany)). Extracts were eluated with methanol at a flow rate of 2 ml/min and monitored at 280 nm (Nylund and Wallander, 1992). For further details on the extraction procedure, see Olsrud et al. (2004).

Hair roots were visually examined for ErM colonization using the staining method of Phillips and Hayman (1970), followed by visual examination according to the magnified intersections method described by McGonigle et al. (1990). In order to get a representative subsample of hair roots for visual examination, all roots were spread evenly over a four segmented Petri dish from which one segment was randomly chosen. All roots that were >2 mm long and had ⩾50% of its length within the borders of that segment were mounted in glycerin on microscope slides and covered with coverslips. Between 4 and 7 slides were used for each sample depending on the amount of roots within the Petri dish segment. Roots were aligned in five rows parallel to the long axis of the slides and observed at 200× magnification. Eight passes, with 0.5 cm intervals across each slide, perpendicular to its long axis, were done. All intersections between roots and the vertical eyepiece crosshair, i.e., 160–200 intersections per sample were considered, except when epidermal cells were missing. The plane of focus was moved completely through the root, and it was noted whether the vertical crosshair cut any ErM hyphal coils (Massicotte et al., 2005) or DSE hyphae (Yu et al., 2001). Only scores on stained hyphal coils that filled up the entire plant cell were counted in order to minimize the subjectivity of the visual determination. Colonizations with a loose net of thick melanized intercellular and intracellular hyphae, as well as microsclerotia but absence of intracellular fine coils, were rated as DSE fungi in the roots (sensu Jumpponen and Trappe, 1998; Yu et al., 2001) while more delicate fungal structures, such as thin hyphae and thin hyphal coils, were classified as ErM fungi (sensu Read and Kerley, 1995; Massicotte et al., 2005). All slides were examined in a random order with the identity hidden from the observer.

No relationship could be found between ergosterol content in hair roots and the proportion of root length containing hyphal coils, i.e., ErM colonization in corresponding root samples ($r^2 = 0.02$, n.s.) (Fig. 1). The concentration of ergosterol in hair roots was significantly positively correlated with the proportion of root length containing DSE hyphae, i.e., DSE hyphal colonization ($r^2 = 0.44$, $P < 0.01$) (Fig. 2).

According to the results presented in this study, ergosterol cannot be used to estimate ErM colonization in hair roots collected from the field (Fig. 1). Ergosterol is a major component of fungal membranes and should therefore correlate with fungal biomass (Nylund and Wallander, 1992). However, in arctic and boreal dwarf shrubs, fungal biomass implies both ErM fungi and DSE fungi. All ericaceous plant species included in this ecosystem study were reported to be colonized with the DSE fungus *Phialocephala fortinii* by Wang and Wilcox under natural conditions (Jumpponen and Trappe, 1998). The positive relationship between ergosterol content in hair roots and the amount of DSE hyphae (Fig. 2) (likely dominated by hyphae from *P. fortinii* (see below)) suggests that ergosterol might be used as a proxy for DSE hyphal colonization,

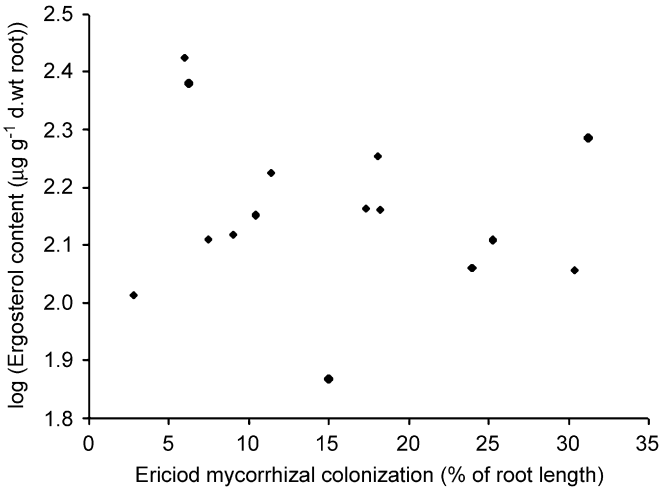

Fig. 1. The relationship between ergosterol content in hair roots and the proportion of root length containing hyphal coils, i.e., ErM colonization, in corresponding root samples ($r^2 = 0.02$, n.s.) (linear regression analysis, SPSS 14.0).