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Mycobioindication method simplified: Sclerotia of *Cenococcum geophilum* Fr. as indicators of stress in forest soils

Melita Hrenko [1], Barbara Štupar [1], Tine Grebenc [1], and Hojka Kraigher [1]

[1] Slovenian Forestry Institute, Večna pot 2, 1000 Ljubljana, Slovenia Affiliation and Address

Contact: Melita Hrenko E-Mail: melita.hrenko@gozdis.si

ABSTRACT

Cenococcum geophilum Fr., an ubiquitus ectomycorrhizal fungus, forms frequent and detectable ectomycorrhiza and sclerotia in temperate forests. It shows proliferation in stress conditions, in situations in which other types of ectomycorrhizae usually diminish in abundance. We tested its applicability as an indicator of the chronic increased ambiental ozone-induced stress. In our experimental system beech seedlings were planted into 20 containers (6 plants per 30 L container) filled with soils from the plot and exposed for two vegetation periods in the sun part of crowns (on towers) or in the shadow (at ground level) in the Kranzberg ozone fumigation system (www.casiroz.de). At the end of the second vegetation period twelve 2x2x2 cm^3 substrate cubes at every 2 cm depth were taken in four replicates per sun-exposed container, from which five were from the fumigated $2xO_3$ and three from the control 1xO3 fumigation system. The total number of sclerotia in 2 cm depth cubes in the volume of ca. 8 cm^3 per depth layer was higher in the fumigated containers (at $P<0.001$), and their distribution showed a statistically significant difference in the peak number of sclerotia in the upper 2-8 cm layers (Fisher LSD at $P<0.001$, comparisons for factor ozone within each depth layer). Their numbers have shown a clear distribution along depth layers and differed among the fumigated and ambient ozone exposed containers. We propose that *C. geophilum* can be considered as an applicable bioindicator of air pollution in different forest ecology studies.

KEYWORDS: mycobioindication, *Cenococcum geophilum* Fr., sclerotia, ozone, drought, stress

1. INTRODUCTION

Cenococcum geophilum Fr. is a a cosmopolitan ectomycorrhizal fungus, it has an extremely wide host and habitat range, and is recognized as a pioneer species in different soil substrates and extreme ecosystems (LoBuglio 1999). The persistence of its sclerotia in soil for several years is a reservoir of fungal inoculum for new seedlings re-establishment and can provide means of overcoming hostile environmental periods. The occurrence of *C. geophilum* mycorrhizae or sclerotia have been among first ectomycorrhizal types suggested for application as bioindicators of stress (Kowalski 1987); however, in mycorrhizal inoculum potential studies their abundance was equally high in polluted and non-polluted substrates or forest research plots (Kraigher et al. 1996). To support the proposed bioindicative value of *C. geophilum* we studied types of ectomycorrhiza in adult beech trees (Grebenc and Kraigher 2007) and in young beech plants grown in containers (Železnik et al. 2007) in an ozone-fumigation experiment, and the occurrence of its sclerotia in containers, planted with young beech plants, aiming to assess ozone stress-related below-ground differences among the fumigated and control plants.

2. MATERIAL AND METHODS

Beech seedlings were planted into 20 containers (6 plants per 30 L container) filled with soils from the plot and exposed for two vegetation periods in the sun part of crowns (on towers) in the Kranzberg ozone fumigation system (www.casiroz.de). At the end of the second vegetation period twelve 2x2x2 cm^3 substrate cubes at every 2 cm depth were taken in four replicates per sun-exposed container, from which five were from the fumigated $2xO_3$ and three from the control

1xO3 fumigation system. *C. geophilum* sclerotia counts were analysed by SigmaStat3.1 and presented by SigmaPlot9.0. Two-way analysis of variance including Fisher LSD method for all pair wise multiple comparison procedures were applied per treatment and depth layer for number of sclerotia.

3. RESULTS AND DISCUSSION

The total number of sclerotia in 2 cm depth cubes in the volume of ca. 8 cm^3 per depth layer was higher in the fumigated containers (at $P<0.001$), and their distribution (Figure 1) showed a statistically significant difference in the peak number of sclerotia in the upper 2-8 cm layers (Fisher LSD at $P<0.001$, comparisons for factor ozone within each depth layer). Their numbers have shown a clear distribution along depth layers and differed among the fumigated and ambient ozone exposed containers. We propose that comparative occurrence of *C. geophilum* sclerotia can be considered as an applicable bioindicator of air pollution in different forest ecology studies.

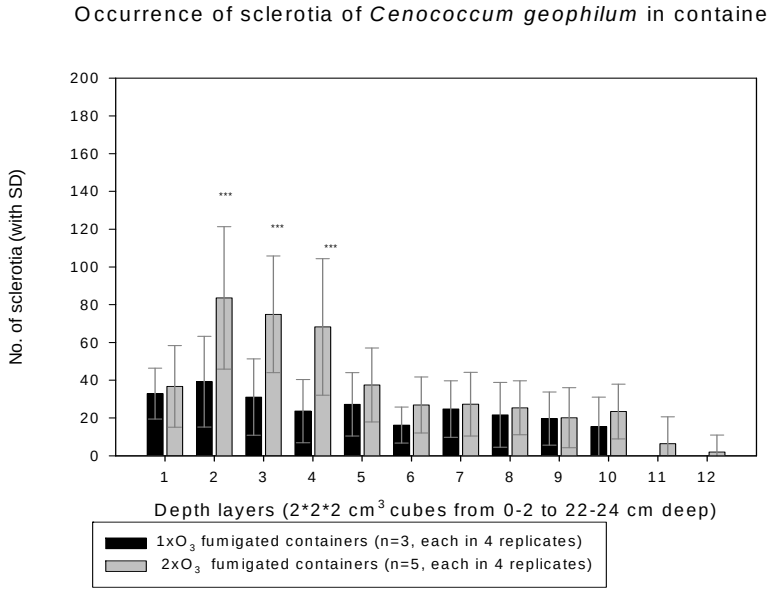

Figure 1. Number of sclerotia of *Cenococcum geophilum* in 2 cm substrate layers in ambient and double-O_3-fumigated experimental set-up, *** marks statistically significant difference ($P<0,001$) as tested by Fisher LSD method)

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