QUANTIFICATION OF ROOT FUNGI USING SIGNATURE FATTY ACIDS

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Table 1. Specificity of selected fatty acids among common root inhabiting microbes. Green cell refers to presence of the respective fatty acids in the different root inhabiting microbes (Larsen et al 1998; Larsen et al 2000)

Root microbe	Kingdom	Charateristic	Specificity of selected signature fattty acids									
			14:0	14:1w9	16:1w5	16:1w7	16:0	18:2w6	18:1w9	18:1w7	20:4	20:5
Spongospora subterranea	Protista	Virus vector					■	■	■		■	■
Plasmodiophora brassica	Protista	Club root									■	■
Aphanomyces euteiches	Chromista	Root pathogen	■	■		■	■	■	■		■	■
Pythium spp.	Chromista	Damping-off	■			■	■	■	■		■	■
Phytophthora spp	Chromista	Root pathogen	■			■	■	■	■		■	■
Glomus spp	Eumycota	Mycorrhiza			■	■	■	■	■	■	■	■
Fusarium spp	Eumycota	Root pathogen					■	■	■			
Rhizoctonia spp	Eumycota	Root pathogen					■	■	■	■		
Plants	Planta	Host	■			■	■	■	■			

Background

Both deleterious (pathogenic) and beneficial (mycorrhizal) fungi inhabit plant roots with strong impact on plant growth and health. Various methods have been used to quantify these fungi, such as disease index, staining techniques, serological/genetic/biochemical markers and indirect measurements of plant parameters. The objective of this work is to evaluate the possibility of using signature fatty acids to quantify root-inhabiting fungi *in planta*.

Fatty acid methodology

Different fatty acid-based methods can be used to quantify fungi. Membrane bound phospholipid fatty acids (PLFA) can be used for biomass estimation and neutral lipid fatty acids (NLFA) for estimation of fungal energy reserves. The NLFA/PLFA ratio provides information on the physiological status of the fungus. The PLFA/NLFA method is, however, quite laborious, and if it is of minor importance to distinquish between PLFA and NLFA, the whole cell fatty acid (WCFA) analysis, which is much faster, can be used as an alternative to give information of root infection intensity.

Results and discussion

Signature fatty acids have been used to quantify arbuscular mycorrhizal fungi (16:1ω5) and the pea root pathogen *Aphanomyces euteiches* (14:1ω9) (Larsen et al, 2000; Larsen & Bødker, 2001) in pot- and field experiments. In pot experiments, we have further used arachadonic acid (20:4) to estimate root infection intensity of the plasmodiophorids *Plasmodiophora brassica*, causing club root in cabbage (Fig. 3) and analyses of the fatty acid profile of resting spores of the virus vector *Spongospora subterranea*, revealed high amounts of arachadonic acid.

Conclusions

- Signature fatty acids offer the possibility of measuring biomass, energy reserves and root infection intensity of various root-inhabiting microbes *in planta*.
- 14:1w9 and 16:1w5 seems to be highly specific to *Aphanomyces* and *Glomus,* respectively. Can be used in both field and greenhouse studies.
- 20:4 and 20:5 can be used to quantify root-inhabiting organisms among *Plasmodiophora, Spongospora, Aphanomyces, Pythium, Phytophthora and Glomus* under controlled conditions.
- No obvious fatty acid signatures for *Fusarium* and *Rhizoctonia* (Eumycota) in roots.

Future work

Can signature fatty acids be used to estimate soil inoculum potential of root fungi?

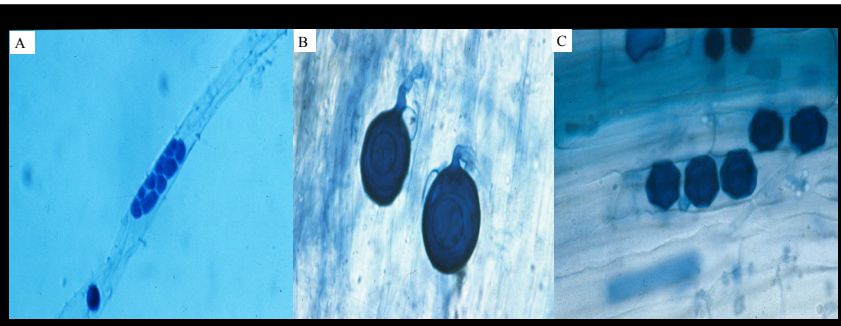

Figure 1. Common root inhabiting fungi revealed after clearing with 10 % KOH and subsequent staining with trypan blue. A) Zoosporagium of *Spongospora subterranea* in tomato root, B) Oopores of *Aphanomyces euteichhes* in pea roots and C) resting spores pf *Olpidium* sp also in pea root.

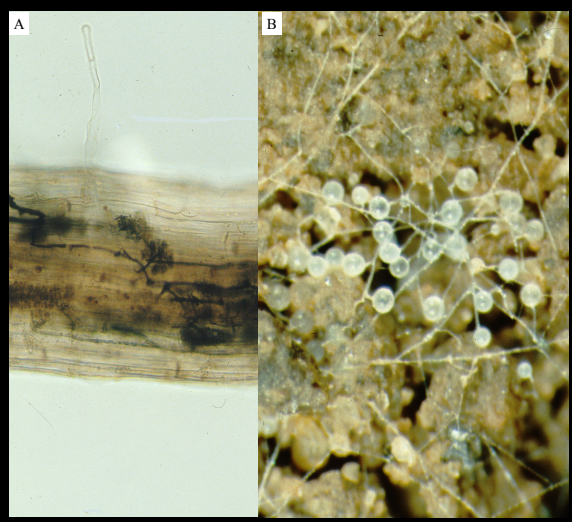

Figure 2. Cucumber root with internal (A) and external (B) mycelium of the AM fungus *Glomus intrradices*

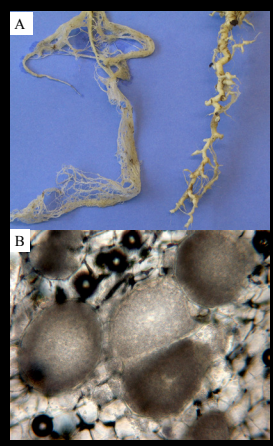

Figure 3. Symptoms of club root in chinese cabbage (A). To the left healthy root system and to the right clubbed roots. (B) Giant cells in clubbed roots of chinese cabbage containing resting spores of *Plasmodiophora brassica*.

Literature
Larsen J, Olsson PA & Jakobsen I. 1998. *Mycological Research* 102: 1491-1496.
Larsen J, Mansfeld-Giese K & Bødker L. 2000. *Mycological Research* 104: 858-864.
Larsen J & Bødker L. 2001. *New Phytologist* 149: 487-493.