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Establishment, persistence and effectiveness of arbuscular mycorrhizal fungal inoculants in the field revealed using molecular genetic tracing and measurement of yield components

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Summary

- Inoculation of crop plants by non-native strains of arbuscular mycorrhizal (AM) fungi as bio-enhancers is promoted without clear evidence for symbiotic effectiveness and fungal persistence. To address such gaps, the forage legume *Medicago sativa* was inoculated in an agronomic field trial with two isolates of *Funneliformis mosseae* differing in their nuclear rDNA sequences from native strains.
- The inoculants were traced by PCR with a novel combination of the universal fungal NS31 and Glomeromycota-specific LSUGlom1 primers which target the nuclear rDNA cistron. The amplicons were classified by restriction fragment length polymorphism and sequencing.
- The two applied fungal inoculants were successfully traced and discriminated from native strains in roots sampled from the field up to 2 yr post inoculation. Moreover, field inoculation with inocula of non-native isolates of *F. mosseae* appeared to have stimulated root colonization and yield of *M. sativa*.
- Proof of inoculation success and sustained positive effects on biomass production and quality of *M. sativa* crop plants hold promise for the role that AM fungal inoculants could play in agriculture.

Introduction

Recently, considerable attention has been paid to the management of soil biota as providers of key ecological services (Myers, 1996; Barrios, 2007). Such organisms of particular anthropogenic interest are often referred to as 'ecosystem engineers' and 'biofertilizers'. Indeed, more and more biota are deliberately released to the environment with the aim of restoring ecosystems, combating pollution and pests, or profiting from plant growth-stimulating effects of root–microbial symbioses. One of the most important plant–microbe mutualisms is the association formed between plant roots and arbuscular mycorrhizal (AM) fungi of the phylum Glomeromycota. The large majority of land plants, including many important agricultural fodder and grain crops, form arbuscular mycorrhizas (Smith & Read, 2008), whose main beneficial effect for plants lies in much improved soil exploitation by the extraradical hyphal network (Giovannetti *et al.*, 2001; Avio *et al.*, 2006). Active uptake, translocation, and transfer of mostly poorly plant-available soil mineral nutrients, such as phosphorus (P) and zinc (Zn), by these fungi are thought to make the greatest contributions to improved plant growth (Smith & Read, 2008), in addition to contributions to soil aggregate formation and protection of their host plants against biotic and abiotic environmental stressors (Newsham *et al.*, 1995). Based on their supportive function in plant nutrition, AM fungi are often imprecisely referred to as 'biofertilizers', but unlike N_2-fixing rhizobia, they do not actually contribute new mineral nutrients and thus are better referred to as 'bio-enhancers' of plant performance. Bio-enhancement through deliberately released AM fungal strains could profitably be used in low-input agriculture, provided that the fungal inoculants are effective in promoting crop yield and quality and are able to persist among local residents of the indigenous AM fungal assemblages.

Initiatives towards improvements in low-input and organic farming have attempted to implement agricultural management strategies favouring crop plant-beneficial AM fungi. In recent years, much effort has been dedicated to finding suitable formulations for AM fungal propagules and appropriate means for their application to the field (Gianinazzi & Vosatka, 2004). Such efforts seem justified, as a recent meta-analysis showed that plant biomass production and P uptake in the field are usually positively correlated with AM fungal root colonization, which can