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Letter to the Editor: The formation of arbuscular mycorrhizae by an Ascomycete?

Stefan Hempel

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A recent publication by Fan et al. (2008) in *Biotechnology Letters* (30:1489–1494) described the isolation of *Penicillium pinophilum* (anamorphic Ascomycota) from strawberry (*Fragaria* × *ananassa* Duch. CV. Zoji) roots and reported the effects of re-inoculation with pure cultures of *P. pinophilum* on the growth and nutrient uptake of strawberry plants. The authors state that the isolate of *P. pinophilum* formed arbuscular mycorrhiza, a symbiosis known to be established between the majority of terrestrial plant roots and fungal species of Glomeromycota (Schüßler et al. 2001), yet never with fungi outside of this phylum. Glomeromycota, also known as arbuscular mycorrhizal fungi (AMF) are obligate symbionts, so far known to be fully dependent on carbon delivered from their host plants, which they in turn supply with limiting nutrients from the soil, e.g. phosphate (Smith and Read 1997). It is therefore unlikely that Fan et al. (2008) really isolated an AMF species, as their isolate grew on Melin–Norkans (MMN) media in absence of any host roots. On the other hand, as the authors identified their isolate morphologically and by molecular methods, providing a sequence in GenBank (accession no. EU277738), I have no doubt that their culture at least contained a saprotrophic fungus able to grow on artificial media, and likely this was the dominant, prolifically growing fungus in their culture.

The species *P. pinophilum* has some degrading capabilities (e.g. Rando et al. 1997) and was characterized as a "minor pathogen" causing growth retardation, e.g. in tomato (Gamliel and Katan 1993). There are also studies describing antagonistic effects of *P. pinophilum* against plant pathogenic fungi (e.g. Alagesaboopathi 1994). However, no study so far has reported positive effects on plant growth and nutrient capture as were observed by Fan et al. (2008).

It is noteworthy that several publications reported synergistic effects of AMF and *Penicillium* spp. (e.g. Babana and Antoun 2006; Cabello et al. 2005; Chandanie et al. 2006; Zaidi and Khan 2007) on plant growth, even specifically involving *P. pinophilum* (Gryndler et al. 2002b). In addition, strawberry was repeatedly reported to react positively to AMF inoculation (e.g. Gange 2001; Gryndler et al. 2002a). It is therefore likely that the increase in biomass as well as nitrogen and phosphate content of the strawberry plants observed in the study by Fan et al. (2008) was potentially due to undetected contamination of their pots with AMF. This hypothesis is supported by the AMF colonization rates reported in the study. The colonization of plant roots by AMF can be easily identified by the characteristic formation of arbuscules, tree-shaped branching structures that invaginate the plant cell plasmalemma and create a large area of membrane–membrane

S. Hempel (✉)
UFZ, Helmholtz Centre for Environmental Research, Theodor-Lieser-Straße 4, 06120 Halle, Germany
e-mail: hempel.stefan@gmail.com

apposition contact (see Vierheilig et al. 2005). Such structures were also clearly visible in the roots of the strawberry plants (personal communication).

The question remains how the contamination could have occurred as Fan et al. (2008) used autoclaved soil as growth substrate and inoculated at least one of their treatments with fresh hyphae from the culture material of *P. pinophilum* which, due to the obligate biotrophism of Glomeromycota, could not have any AMF propagules. A contamination in the greenhouse is also unlikely as the non-inoculated control plants were found to contain no AMF structures. However, due to the stimulatory effects saprobic fungi can have on spore germination and hyphal growth of AMF (Fracchia et al. 2004) it is conceivable that inoculation with *P. pinophilum* stimulated AMF propagules surviving the autoclaving, and thus the AMF symbiosis was established. In case of the non-inoculated controls this stimulatory effect was not present and therefore the roots were not colonized by AMF.

Given the facts presented here, the finding that *P. pinophilum* is capable to form arbuscular mycorrhizal symbiosis should be critically tested in further experiments with other plants in order to confirm or confute the results. Simple experiments on the resistance of AMF propagules to extreme conditions encountered during autoclaving should also be undertaken.

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