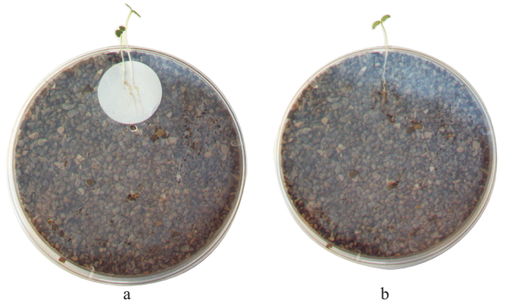


a b

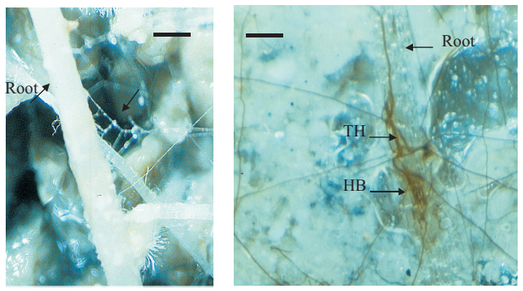
Fig 1 Inoculation and planting of seedlings in the Petri-dish microcosm: a, inoculation with pre-germinated spores on millipore paper; b, spore directly inoculated onto the root of *Desmodium ovalifolium* seedling.

RESULTS

This system was effective for *G. manihotis* BEG112, *G. rosea* BEG111 and *S. heterogama* BEG40. However, due to the low germination percentage and consequent problems of contamination, pre-germinated spores were not used for *A. tuberculata* BEG41. Instead fresh crude inoculum, containing spores, mycelia and root fragments, from six-month-old pot-cultures of the fungus was used to fill the 1/3 volume of the 18 cm diameter Petri-dish around the root of the plant. Another alternative tried was to transplant *D. ovalifolium* (6-month-old) colonised by the fungus into 18 cm diameter Petri-dish containing the medium. Using this modification *A. tuberculata* BEG41 could be established in the Petri-dish system.

The ease of use and simplicity of the Petri-dish system allowed the early stages of development of the AMF symbiosis to be studied. In all four AMF, spore germination, root colonisation and development of the ERM, and spore ontogeny could be observed.

Root Colonisation. The germinating hyphae in the direct-inoculated treatments did not directly colonise the root but grew in random directions, often away from the roots. The entry points formed by *S. heterogama* BEG40



a b

Fig 2 Root colonisation: a, The extra radical mycelium of of *Glomus manihotis* BEG112 formed a dichotomous branching (yellow) when approached a root. b, The hyphae of *Scutellospora heterogama* BEG40 thickened and pigmented (TH) prior to the formation of entry points, and produced intensive hyphal branches (HB) around the entry points. The bar represents 100 µm.

were the easiest to observe due to their colour (pigmented brown) and the fact that they formed a distinct intensive proliferation of hyphae on the surface of roots (Fig 2). Colonisation of the roots by pre-germinated spores was not detected until 2-3 weeks for *G. manihotis* BEG112 and *G. rosea* BEG111, 3-4 weeks for *A. tuberculata* BEG41 (inoculated with a crude inoculum), and 6-7 weeks for *S. heterogama* BEG40. Successful colonisation resulted in the growth of ERM. Where millipore inoculum was used, this was particularly evident as the extension of mycelium beyond the millipore paper. If colonisation was not successful, there were no signs of mycelium growing beyond the millipore paper.

The Architecture of the Extra-Radical Mycelia (ERM). In all cases, the ERM comprised germ-tubes, infection networks, runner hyphae, hyphal networks and hyphal bridges (terminology *sensu* Friese and Allen 1991)

Table 1 Time (days after inoculation) for spores of different arbuscular mycorrhizal fungi (AMF) species to germinate on either millipore paper or on roots

AMF species	On millipore paper (dai)	On root (dai)
Acaulospora tuberculata BEG41	12± 5*	nd
Gigaspora rosea BEG111	6± 2	17±5
Glomus manihotis BEG112	4± 1	18±5
Scutellospora	11 ±4	>144

*Most of these germinated spores were later contaminated by either bacteria or other fungi and were not used for futher experimentation. nd, no data, *A. tuberculata* BEG41 was inoculated using crude inoculum or transplanted seedling colonised by this fungus. dai, day after inoculation.

in the microcosm. Runner hyphae either grew away from the root, or grew alongside the colonised root. The former type of runner hypha of *A. tuberculata* BEG41 and *G. manihotis* BEG112 formed a hyphal network in the substrate and subsequently produced infection networks when approaching another root (Fig 2). Many branches of the runner hyphae passed over the root and did not attempt to penetrate. The runner hyphae that grew alongside the colonised root formed entry points on the same root and also produced branches that formed a hyphal bridge that connected adjacent roots and colonised them.

The diameter and length of the hyphae formed by AMF varied among species (Table 1). *Glomus manihotis* BEG112 formed significantly thicker and longer hyphae compared with *A. tuberculata* BEG41, *G. Rosea* BEG111 and *S. heterogama* BEG40. The colour of the hyphae was generally hyaline, except for *S. heterogama* BEG40 which had dark red-brown hyphae main channel hyphae and hyaline ephemeral hyphae (Fig 3).

Short and fine intensive hyphal branching ("arbuscule-like" [ALS] or later called branched absorbing structure [BAS] (*sensu* Bago *et al*. 1998a, b) was formed at the tips of hyphal branches in all AMF, but the shape and size varied (Fig 3). *Acaulospora tuberculata* BEG41 formed more intensive and shorter branching type compared with *G. manihotis* BEG112, *G. rosea* BEG111 and *S. heterogama* BEG40. The BASs were also found around newly formed