structures, iii) reproducible, and iv) requiring simple facilities which can be adopted by most laboratories. Four species of AMF belonging to four different genera were tested.

MATERIALS AND METHODS

Media Preparation. Terragreen, an inert attapulgite clay (Oil Dri, Wisbech, Cambs, UK) was washed with tap water then soaked overnight in 5 mM MES (2-[N-Morpholino] ethanol-sulfonic acid) buffer solution (Vilariño *et al.* 1997). The Terragreen was autoclaved at 121°C for 20 min then was distributed into 9 cm diameter Petri-dishes. A small hole (0.5 x 0.5 cm) was made at one side of each Petri-dish to insert the seedling.

Seed Surface-Sterilisation and Germination. Seeds of *Desmodium ovalifolium* were surface-sterilised by immersing the seeds in 95% (v/v) ethanol for 10 sec and then soaking them in 3% (v/v) H_2O_2 for 5 min. Finally, the seeds were rinsed three times with sterile distilled water (dH_2O). To break dormancy, the seeds were soaked in sterile boiling water for 5 mins and then in sterile cold dH_2O overnight. The seeds were germinated on fine terragreen (1-2 mm diameter particles) in a 250 mL glass beaker and kept in a growth chamber, Fitotron (Gallenkamp, UK) maintained at 30°C, 12 h light at 300 µmol m^{-2} s^{-1} and 12 h dark, at RH 60%.

Spore Extraction and Surface-Sterilisation. Spores of *A. tuberculata* BEG41, *G. rosea* BEG111, *G. manihotis* BEG112 and *S. heterogama* BEG40 were extracted from pot-cultures by sieving using a 500 µm pore diameter sieve placed on top of a 63 µm pore size sieve. The contents of the 63 µm sieve were subjected to sucrose gradient centrifugation (http://wwwbio.ukc.ac.uk/beg), washed and placed in 1.5 mL sterile eppendorf tubes. They were centrifuged for 2 min at 13 000 g using a microcentrifuge model 1-15 (Sigma, Germany) to pellet the spores and remove excess water. One mL of 5% v/v commercial chloros (Hays Chemical Distribution Ltd., UK) containing >5% and <16% chlorine was pipetted into the tube and left for 2 min. The eppendorf was then centrifuged to pellet the spores and the sterilant removed. Finally, spores were washed five times with sterile dH_2O.

Spore Germination and Plant Inoculation. The sterilised spores were germinated on millipore papers, Pragopor (Pragochema ltd., Prague), pore size 0.40 µm, diameter 2.5 cm, according to the method described by Brundrett and Juniper (1995) with some modification. Spores were germinated on millipore papers in 9 cm diameter Petri-dishes containing half volume of moist sterile fine-terragreen (1-2 mm diameter), 5 spores per millipore paper and 10 millipore papers per dish. Two replicates were set-up for each species of AMF. The spores were then incubated at 30°C in the dark and checked every two days for 20 days. Subsequently, the spores, together with the millipore paper, were inoculated onto the seedling (the tap root of the seedling was put on the millipore paper as close as possible to the hyphal tip) and planted in the prepared Petri-dishes (Fig 1a). For direct spore inoculation, five sterilised spores of the AMF species were arranged on the tap root of the seedling close to the tip, and the seedlings were then planted in the Petri-dish (Fig 1b). The Petri-dish was sealed with PVC tape leaving the side of the dish opposite to the hole for the plant un-taped. The Petri-dishes were then arranged vertically in square plastic containers containing water, such that the water would be absorbed by the medium to keep it moist but not waterlogged. Cultures containing different species of AMF were kept in separate boxes to avoid cross-contamination. The systems were placed in the greenhouse (min 15°C/max 40°C, relative humidity 60-80%, light intensity 400-600 µmol $m^{-2}s^{-1}$). Water was added to the plastic container as needed and fed fortnightly with 1.4 g l^{-1} Vitafeed 102 fertiliser (Vitax Ltd., Leicester, UK) containing 18% N, 0% P, and 36% K with trace elements. Feeding was conducted by replacing the water in the container with the fertiliser solution. Checking for mycorrhizal development, including colonisation and sporulation was conducted weekly for the first four months and then monthly for another six months by direct observation of the microcosm under a dissecting microscope (Nikkon SMZ-U) at 15 - 100x magnifications.

Measurement of Growth Parameters of AMF. Ten months after inoculation a sampling was conducted to measure spores diameter, hyphal diameter and length. Twenty five spores per AMF species from 5 Petri-dishes were mounted in water on a microscope slide, covered with a cover slip, and measured under a compound microscope at x 100 (Axioskop, Zeiss, Germany). The hyphal diameters were determined from 50 randomly selected fragments of hyphae directly picked up from the Petri-dish microcosm under a dissecting microscope (Nikkon SMZ-U) at 20x magnification and mounted in water as for spores. Measurement was conducted under a compound microscope at x 1000 (Axioskop, Zeiss, Germany). To measure total hyphal lengths, 4 cores (1 cm diam.) were taken from 4 random positions in each Petri-dish which were later mixed and used as a composite sample. Five replicates were used for each AMF species. The samples were agitated in 100 ml of deionised water (dH_2O) by stirring for 1 min at 200 rpm and allowed to settle for 10 sec. Five mL of the solution containing hyphae was then filtered onto Pragopore membrane filters (25 mm diam. and 0.45 µm pore size). The membrane filters, containing hyphae, were transferred to microscope slides, and subsequently two drops of 0.05% Trypan blue in lacto-glycerol (lactic acid:glycerol:dH_2O; 5:1:1) were added to the filter. The entire membrane was scanned under a compound microscope (Axioskop, Zeiss, Germany) for mycelium and estimations of mycelial length were made using an eyepiece graticule at x 100. The length of mycelium was expressed in meters of total hyphae in 1 gram of dry substrate (oven-dried for 3 h at 105°C).