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P.A. McGee · V. Torrisi · G.S. Pattinson

The relationship between density of *Glomus mosseae* propagules and the initiation and spread of arbuscular mycorrhizas in cotton roots

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Abstract The experiments aimed to determine the relationship between density of propagules in soil and initiation and spread of an arbuscular mycorrhizal fungus in the cotton roots. As few as 10 propagules of *Glomus mosseae* in approximately 95 g soil located in a band 25 cm below the soil surface established mycorrhizas in more than 80% of cotton roots at the point of inoculation within 36 days. Secondary spread was initiated 10–13 days after primary colonisation in treatments inoculated with one, 10 or 100 propagules. Spread of mycorrhizas within the root system was rapid from 100 propagules and was slower with fewer propagules.

Key words AM fungi · Inoculum density · Initiation of AM · Spread of AM · Cotton

Introduction

The rate and extent to which root systems become colonised by arbuscular mycorrhizal (AM) fungi is important for production of highly dependent crops such as cotton (Rich and Bird 1974). Colonisation rates are influenced by the placement and density of AM fungi propagules in soil, edaphic factors such as soil type and fertility, climatic factors such as soil temperature and moisture availability, host factors such as species and age of roots, and the fungal species (Abbott and Robson 1984; Hepper 1985; Amijee et al. 1986, 1989).

In soils used to grow cotton, densities of AM fungi decline down the profile (Nehl et al. 1999). When AM fungi are depleted in the upper profile (e.g. long fallow disorder, Thompson 1987), colonisation relies on pockets of AM fungi below the surface layer (Nehl et al. 1999). Colonisation spreads rapidly through the root system from pockets of inoculum when high densities of propagules are present initially (Torrisi et al. 1999); however, initiation and spread may be slowed or limited at lower densities.

Previous studies of the relationship between density of propagules and colonisation rates indicate a direct relationship up to the point where colonisation reaches a plateau. In those experiments, trap plants were sown into inoculum which was either mixed through sterile soil (e.g. Smith and Walker 1981; Sanders and Shiehk 1983) or placed as a cylinder of inoculated soil within sterile soil (Wilson 1984). A density of 1–5 propagules g^{-1} soil was found to be necessary to attain a high rate and level of colonisation.

Interpretation of these studies is complicated by at least three factors: pot shape, variation in energy reserves of propagules, and secondary spread. Roots normally elongate down the soil profile. Root growth in shallow experimental pots becomes circular soon after elongation commences, and roots proliferate within 6 weeks following initiation of colonisation (Torrisi et al. 1999). This increases the chance of contact between the symbionts in the experimental pots, artificially reducing the density at which colonisation reaches a plateau. Use of spores as inoculum increases chance of contact due to greater organic reserves in spores than in hyphae, thereby influencing primary colonisation and the rate of spread of colonisation (Wilson 1984). Higher rates of germination have been observed for spores from pot cultures than from field soil (McGee et al. 1997) and thus data based on spore inoculum must be interpreted cautiously. Finally, secondary spread of colonisation may be confused with primary colonisation in confined root systems. These difficulties of interpretation led us to consider a different experimental approach.

We wish to develop a predictive model of production based on field densities of AM fungi, where the size of the fungal population may change dramatically (Thompson 1987; Pattinson and McGee 1997). Thus our aim here was to clarify the relationship between the

P.A. McGee (✉) · V. Torrisi · G.S. Pattinson
School of Biological Sciences, University of Sydney,
NSW 2006, Australia
e-mail: peterm@bio.usyd.edu.au
Fax: +61-02-9351 4771

density of propagules and the rate of initiation and spread of colonisation in cotton roots.

Materials and methods

Use of field soil as inoculum is problematic due to the large variation in fungal species and density of propagules. We, therefore, chose to use a single fungus (*Glomus mosseae* isolate NBR 4.1, Pattinson et al. 1997). The fungus is common at the site at which we completed controlled experiments and soil from the site has been used in many other experiments (McGee et al. 1997; Pattinson and McGee 1997; Pattinson et al. 1997; Torrisi et al. 1999; Nehl et al. 1999). The fungus was pot cultured in controlled conditions in a growth room with a day length of 13 h at a PFD of 700 μmol m^{-2} sec^{-1} at 25 °C, and a night temperature of 20 °C (McGee et al. 1997). The experimental soil consisted of seven parts coarse sand mixed with three parts clay by volume. A modification of the most probable number (MPN: Porter 1979) was used to quantify the density of fungal propagules. Pot cultures of NBR 4.1 containing roots and other propagules were allowed to dry before being broken apart and sieved through a 2-mm mesh to remove root fragments. While some fine root fragments may pass through the mesh, we found that these are unlikely to contain viable propagules of NBR 4.1 (McGee et al. 1997). Dried root fragments colonised by NBR 4.1 do not initiate mycorrhizas when inoculated onto roots in pot culture (Pattinson and McGee unpublished data). The sieved pot cultures were bulked and mixed manually with autoclaved experimental soil to obtain the desired dilutions.

MPN was determined in 10-cm-tall, enclosed pots containing 570 g total soil moistened to 95% field capacity. A 1-cm band of autoclaved soil was packed at the bottom of the pot, and covered by 95 g of the pot culture mixture, approximately 2 cm deep. The depth was chosen to ensure roots had branched by the time they reached the inoculum and the inoculum was explored thoroughly. The remainder of the pot was filled with autoclaved soil. A 10-fold serial dilution (Wilson and Trinick 1982), replicated five times, was used to determine the number of propagules. A similar volume of inoculum was used in the MPN and the main experiment. Pregerminated seeds of *Trifolium subterraneum* L. cv. Seaton Park were directly sown into each pot, approximately 1 cm below the soil surface. We chose *T. subterraneum* as it has a slower root growth rate than cotton yet maintains similar colonisation characteristics (Pattinson and McGee unpublished data) and so can be grown in shallower pots. Seedlings were thinned following emergence to 10 plants for those harvested at 2 weeks, 6 at 4 weeks and 4 at 6 weeks. We examined the MPN at three harvests because the main experiment was harvested from 12 to 42 days, and the number of propagules initiating colonies may increase over this period (Wilson and Trinick 1982). Pots were placed in the growth room and watered to their initial weight every second day. At harvest, plants were washed free of soil and the roots cleared and stained in Trypan blue (Phillips and Hayman 1970) and examined microscopically for the presence of AM. The MPN was determined (Cochran 1950).

The main experiment determined colonisation from different numbers of propagules. Tubes of soil (Torrisi et al. 1999) were inoculated with a 1-cm band of soil at 24–25 cm below the soil surface containing 1, 10 or 100 propagules, based on the MPN. Moistened soil mix was packed into PVC tubes, 7.9 cm in diameter and 50 cm long, cut in half longitudinally (Torrisi et al. 1999). Two pregerminated seedlings of cotton (*Gossypium hirsutum* cv CS50) were placed in the moistened soil and tubes randomly positioned in the growth room. Cotton was chosen as the trap plant because the tap root elongates predictably down the soil profile, enabling comparison of colonisation at different parts of the root system (Torrisi et al. 1999). The cotton plants were watered every second day with deionised water and fertilised with 20 ml of nutrient solution (Pattinson et al. 1997) every 7 days (Torrisi et al. 1999). Three replicate tubes of each treatment were harvested after 12, 15, 19, 22, 29, 36 and 42 days. These harvests were chosen because previous results (Torrisi et al. 1999) indicated that roots would have been in contact with the band of inoculum for 2 days by the time of the first harvest. Also, mycorrhizas would have spread well beyond the band of inoculum by the last harvest in the treatment inoculated with 100 propagules, thus enabling determination of initiation of secondary spread.

At each harvest, shoots were severed from the plant and weighed. The shoots were then dried at 70 °C for 24 h and reweighed. Transverse sections of soil 2 cm thick were removed and stored at 4 °C until processing some 2–10 days later. Roots from each of 7 sections above and below the inoculum were removed from each treatment at each harvest, weighed, cleared and stained in Trypan blue, and the total length of roots and AM determined microscopically (Giovannetti and Mosse 1984). The remainder of the sections were discarded because of proliferation and coiling at the top and bottom of the tubes, respectively (Torrisi et al. 1999). Data were analysed using either one-way or two-way ANOVA, followed by Tukey's HSD test. Root densities were compared using Student "*t*" test.

Results

A mean of 170 propagules was found in 95 g of inoculant soil at 2 and 4 weeks, and 460 at 6 weeks. We used a mean density of propagules in the sieved potculture of 2 g^{-1} at 2 weeks, enabling dilutions with autoclaved experimental soil to 1, 10 or 100 propagules in the band of inoculum in the following experiment.

AM were first observed in plants inoculated with 10 or 100 propagules after 12 days, and those with one propagule after 29 days (Fig. 1). Colonies were observed in the roots from the band of inoculum and the adjacent segments of soil. Note that AM were absent after 15 days in plants inoculated with 10 propagules. Spread of AM to the zone of roots outside the initial zone of contact was first observed after 22 days for treatments inoculated with 100 propagules, 29 days for treatments with 10 propagules and 42 days for treatments with one propagule (Fig. 1). Each estimate is based on the difference between the appearance of the first colonies within the 5-cm zone of initial colonisation and the section immediately adjacent, while ignoring the first colony formed from 10 propagules.

The mean colonisation of roots in the inoculum was variable (Fig. 2). Colonisation reached a plateau after approximately 15 days in plants inoculated with 100 propagules, and between 29 and 36 days when inoculated with 10 propagules. Colonisation did not reach a plateau in plants inoculated with one propagule during the experiment.

After 42 days, roots appeared denser at the point of inoculation (Fig. 3) for all treatments. In the treatment inoculated with 100 propagules, the sections 5–9, 13–17 were significantly ($P>0.05$) less dense than the inoculated zone. The differences were not statistically significant in treatments with fewer propagules.

Shoot dry weight was not significantly different for each treatment at each harvest. The largest difference was found after 42 days, when the mean dry weight ($\pm$SEM) was 2.78 ± 0.39 in treatments inoculated with

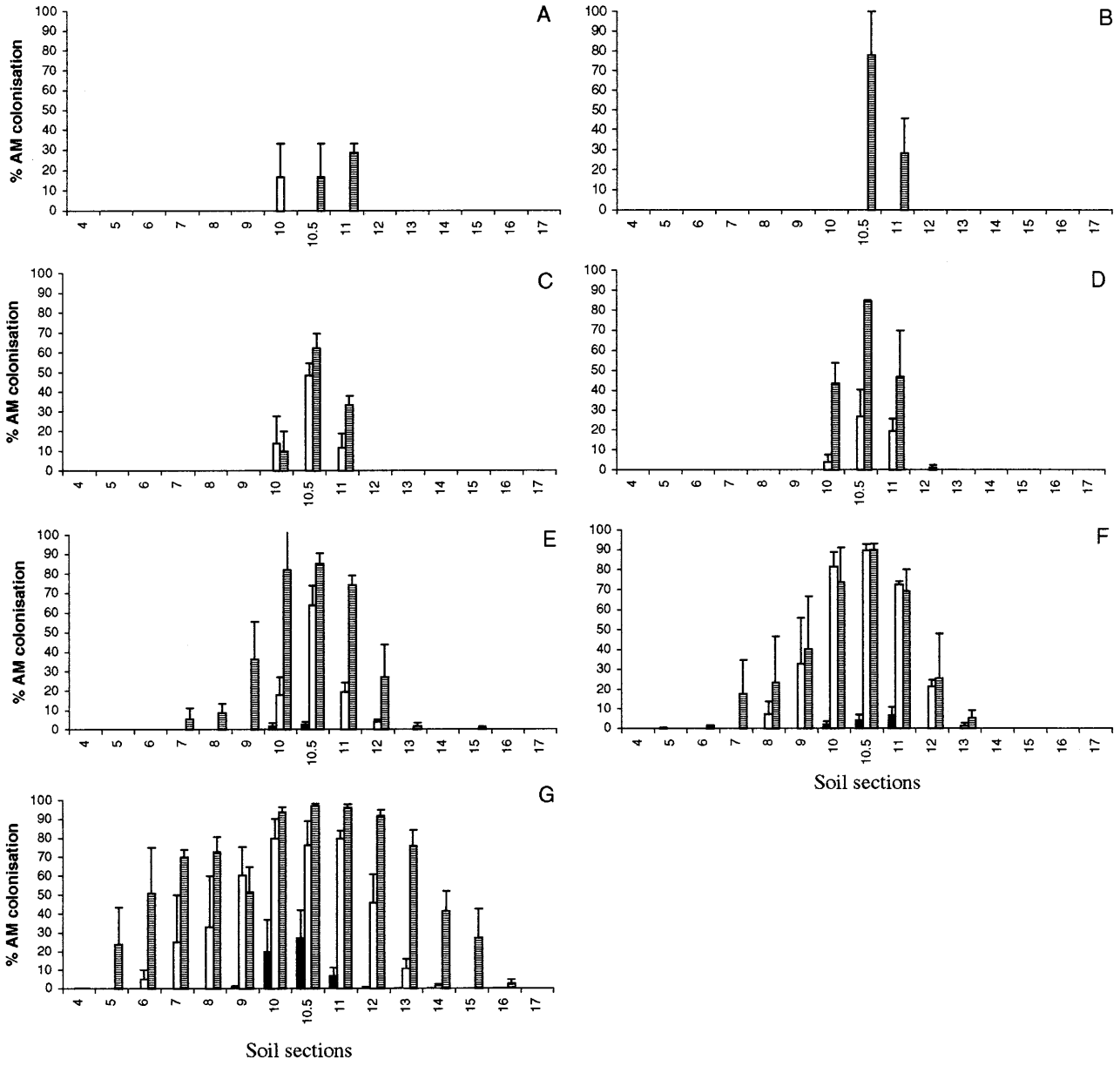

Fig. 1 Mean (±SEM) arbuscular mycorrhizal (AM) colonisation of cotton roots inoculated with 1 (*black*), 10 (*blank*) or 100 (*cross-hatched*) propagules of *Glomus mosseae*, NBR 4.1 above and below the point of inoculation at soil section 10.5 after 12 (**A**), 15 (**B**), 19 (**C**), 22 (**D**), 29 (**E**), 36 (**F**) or 42 (**G**) days

100 propagules, 2.37±0.22 with 10 propagules and 2.15±0.25 with one propagule.

Discussion

The density at which the colonisation plateau is reached in the shortest time, called the critical density (Wilson 1984), is between 10 and 100 propagules, as determined by MPN, distributed through approximately 95 g soil under our experimental conditions. The colonisation of roots growing in 100 propagules reached 85% at the point of inoculation after 15 days. This is similar to the colonisation observed in cotton roots growing in undisturbed cores of soil (Pattinson and McGee 1997) but higher than in *T. subterraneum* growing in undiluted inocula of *G. fasciculatum* (Wilson 1984). Initiation of colonisation of cotton roots growing in 1 propagule in 95 g soil was later, after 29 days. Colonisation did not reach a plateau by the end of the experiment, indicating that this density of propagules was below the critical density.

The critical density of less than 1 g^{-1} in this experiment is lower than previous estimates. Sanders and Shiehk (1983), Smith and Walker (1981) and Wilson (1984) found between 1 and 5 propagule g^{-1}. It is hard to compare these estimates, firstly because the inherent variability of the MPN confounds interpretation of the data. Also, small pots and different experimental condi-

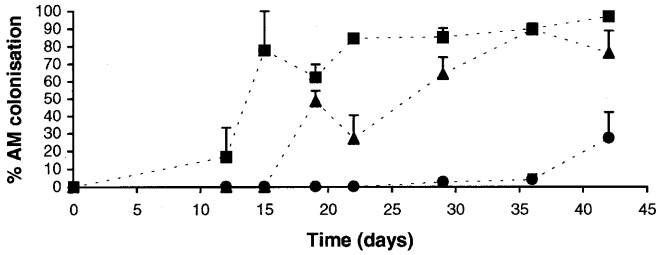

Fig. 2 Mean (±SEM) development of AM colonisation of cotton roots at the point of inoculation, inoculated with 1 ●, 10 ▲ or 100 ■ propagules of NBR 4.1

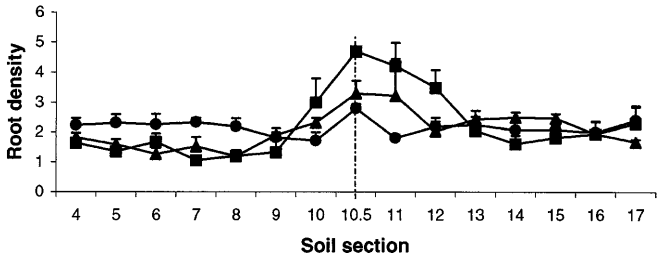

Fig. 3 Root density (cm of roots ml^{-1} soil) of cotton seedlings inoculated with 1 ●, 10 ▲ or 100 ■ propagules of fungus NBR 4.1 after 42 days

tions influence these estimates. The use of tubes in this experiment alleviated most of the effects of pots and the results should more closely reflect conditions in the field. However, tests in the field are necessary to test the relationship.

The data presented above enable us to tentatively estimate densities of propagules in field soil and ultimately to predict growth and production of crop plants. If we assume that similar patterns of colonisation in lab and field soils arise from similar numbers of propagules, then soils used to grow cotton that have been examined had propagules densities in excess of 1 g^{-1} soil at the surface (McGee et al. 1997; Pattinson and McGee 1997) and approximately 0.01 g^{-1} at 1 m depth (Nehl et al. 1999). We have observed up to 200 spores and 0.5 m hyphae g^{-1} soil (McGee et al. 1997), suggesting extremely high densities of propagules at the end of a growing season, in excess of the critical density.

One hundred propagules initiated AM that spread 12 cm both up and down the root system of cotton from the inoculum during the 30 days following initiation. The colonisation from 10 propagules was less and initiation of colonisation was mostly delayed till after 15 days. Ten propagules established 50% colonisation of roots between 19 and 29 days and reached a colonisation percentage similar to 100 propagules after 36 days. This pattern of colonisation is typical of depleted soil (Pattinson and McGee 1997). Colonisation from 10 propagules appeared to be associated with increased variation. The conclusions remain tentative because the treatments were replicated only three times, and the inoculum quantities were based on MPN.

Initiation of colonisation was observed in the zone of inoculum and the immediately adjacent band, presumably because hyphae elongated from the inoculum to the adjacent zone. Thus secondary spread was apparent after 10 days from 100 and 10 propagules and after 13 days from one propagule. The initial colony from 10 propagules is probably a chance event, as the next set of colonies in the treatment was observed 7 days later. Once colonisation commenced, the rate of elongation up and down the root system was apparently not influenced by density of inoculum, though the total length of colonies in each treatment differed markedly in direct relation to the quantity of inoculum (as observed by Walker and Smith 1984). While rapid initiation of colonisation may be associated with stores of energy in propagules, these data suggest that the rate of spread is more directly affected by supply of organic carbon from the host. After initiation of AM, all hyphae would gain similar quantities of carbon because the host has an abundant supply, and thus all hyphae of one isolate would elongate at similar rates, provided the host is receiving abundant PAR.

The data also support the hypothesis of Torrisi et al. (1999) that the initiation of colonisation is followed by proliferation of roots. In this case, the density of inoculum and colonisation were directly related to the increase in proliferation. The cause of proliferation is yet to be determined.

Arbuscular mycorrhizas were initiated rapidly in cotton roots from 100 propagules dispersed through 95 g soil, and mycorrhizas spread rapidly through the roots. Secondary spread was also initiated after approximately 10 days from a range of propagule densities, though initiation of mycorrhizas was delayed and the rate of spread reduced from fewer propagules. The data support the view that the soils used to grow cotton in eastern Australia normally contain huge densities of AM fungi in the surface layers, and that the densities vary significantly over the cycles of crop growth.

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