New Phytol. (1997), **135**, 773–780

Survival of propagules of arbuscular mycorrhizal fungi in soils in eastern Australia used to grow cotton

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(*Received 23 July 1996; accepted 4 December 1996*)

SUMMARY

Soil-borne spores and hyphae of arbuscular mycorrhizal fungi are important propagules in cracking clay soils of northern NSW, Australia. In these soils, senescent roots were uncommon. Although *c.* 4–200 spores g^{-1} soil were found, less than 6% established arbuscular mycorrhizas in trap plants, and this percentage declined over 24 months. Using tetrazolium red as a vital stain, 16–21 % of spores from field soils were found to be viable in fresh soil and 6–7 % after 24 months of storage. Using fluorescein diacetate, the length of stained hyphae of *c.* 0·5 m g^{-1} soil was shown to be halved over 32 wk. The density of viable propagules of arbuscular mycorrhizal fungi in soil declined over time and was reduced by severe disturbance. The fungi that survived to 12 months included a species thought to form dormant spores, while those initiating infection after 24 months, did not.

Key words: AM fungi, survival, vital stains, bioassay, clay soil.

INTRODUCTION

Arbuscular mycorrhizas (AM) are associations between fungi in the Glomales (Zygomycetes) and the roots of plants, in which the fungus functions as an extension of the root system. They are particularly important for plant growth where the soil has low concentrations of phosphate available to the plant and where the plant is dependent on the fungus. The growth of seedlings of dependent plants is determined, in part, by the density of arbuscular mycorrhizal fungi in soil (Abbott & Robson, 1978). In soils used for cropping, the density of arbuscular mycorrhizal fungi is, in turn, dependent on the cultural practices used (Bethlenfalvay, 1992). The host plant is the only source of organic carbon for the fungus. Any disruption of the fungus, or its separation from the source of carbon for extended periods might reduce its density in soil and thereafter reduce benefit to the plant.

Cotton cropping in eastern Australia is characterized by growth of the crop over summer followed by another crop or periods of fallow (no crop). Cotton can be grown in rotation with other summer or winter growing crops, or 'back-to-back' as a monoculture over consecutive seasons. Insufficient rainfall can extend the fallow to more than 12 months, a period associated with a severe reduction in initiation of AM in many crops, in a syndrome called 'Long Fallow Disorder' (Thompson, 1987). Cotton appears to be dependent on AM for maximum rates of plant growth and production of lint (Rich & Bird, 1977; G. S. Pattinson & P. A. McGee, unpublished). These authors indicate that the rate of initiation of AM in the roots influences rates of plant growth and possibly, production of lint.

The rate of initiation of AM is determined primarily by the density of arbuscular mycorrhizal fungi in the soil (Walker & Smith, 1984). The rates of germination and growth of each fungal isolate present are also important. The survival structures of arbuscular mycorrhizal fungi include the hyphal network and spores in the soil, and hyphae and thick-walled vesicles within roots. Survival of arbuscular mycorrhizal fungi has been examined in the southern part of Australia. The fungi appear to survive mainly as hyphal networks (Jasper, Abbott & Robson, 1987; McGee, 1989; Bellgard, 1993), hyphae in dried root fragments (Tommerup &

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Abbott, 1981) and spores. Spores are common in agricultural soils (Mosse & Bowen, 1968; Abbott & Robson, 1977; Hayman & Stovold, 1979) and it is assumed that spores and fungi in root fragments are the main source of infection at the beginning of the growing season in soils used for cropping (Abbott & Gazey, 1994). In undisturbed soils supporting native vegetation, the hyphal network is more important.

These observations concerning fungal propagules are probably valid for southern Australia, where rainfall is highly seasonal, falling mainly in cooler months. Crops are grown and annual native plants complete their life cycle during the period of available moisture. Spore germination is suppressed when soil temperatures are high (Tommerup, 1983*b*; McGee, 1989). Some species of arbuscular mycorrhizal fungi in the family Acaulosporaceae have spores that go through a period of obligatory dormancy before germination (Tommerup, 1983*a*; Gazey, Abbott & Robson, 1993; McGee, unpublished) preventing germination during summer. In southern Australia, the growth periods of fungi and plants appear to coincide and are largely seasonal.

In the cotton growing areas of eastern Australia, the most important propagules to survive the period when host plants are absent are assumed to be spores and fungi in root fragments. The cotton-growing area differs significantly from those studied in southern Australia. Rain can fall at any time, soil temperatures are often high when soil is wet, and plant growth is primarily determined by soil moisture. Further, cotton is usually grown in cracking clay soils, which increasingly fragment during drying, a process that might also fragment the hyphal network in the surface layers. Fungi would be protected from fragmentation if they survived in roots or as spores during drying of the soil. These factors indicate that the hyphal network might be of limited importance to the survival of the arbuscular mycorrhizal fungi in the absence of the host plant.

Fungi under native vegetation might be unaffected by high soil temperatures, and adapted to respond rapidly to moisture. Under crops, the rate of formation of surviving propagules seems less important because of the long time the roots of an irrigated host plant are present. We predict, therefore, that arbuscular mycorrhizal fungi have characteristics that enable the fungi to grow during the period of plant growth: those in cropping systems also having the capacity to survive through cultivation and fallow. We predict survival of the fungi in root pieces and hyphal networks in undisturbed soil. We hypothesize that under irrigated crops such as cotton, a propagule such as a spore that has the potential to germinate several times or has a period of dormancy could be more likely to survive the variable cropping/fallow sequence.

The nature of surviving propagules is important to the management of plant growth. If the nature of propagules and length of survival of each fungal species is understood, densities can be predicted for initiation of AM each growing season. In the cotton-growing industry, efforts are being made to manage the density of arbuscular mycorrhizal fungi in soil in order to maximize production of lint. To achieve this goal, the propagules which initiate AM need first to be understood before they can be quantified. This paper examines the fungal propagules which initiate AM in soils used for growing cotton.

MATERIALS AND METHODS

Soils were collected from: a farm, 'Waverly', 25 km west of Wee Waa, NSW; 'Auscott', Narrabri; the Australian Cotton Research Institute, Narrabri, NSW; and 'Warra', 30 km northwest of Dalby, Queensland. These soils are fertile grey cracking clays with blocky structure (Stace *et al.*, 1968). A red cracking clay loam (Stace *et al.*, 1968) was also collected from 'Millawa', 15 km east of Warren, NSW.

The soil from 'Waverly' was collected as dry blocks during January, 1991 (summer), from a paddock that had never been cultivated and was used for grazing. The dominant vegetation was perennial grass with some annual herbs. Edges of blocks were trimmed to fit open-ended tubes 10 cm diameter, 10 cm deep. The weight of these tubes with dry soil was 894 ± 133 g. A further collection of soil was passed through a hammer mill with a 2-mm sieve to simulate severe disturbance. Approximately 830 g sieved dry soil was placed in similar tubes. A screen was attached to the bottom of each tube to prevent loss of soil and allow aeration of the soil. The rate at which AM formed in trap plants sown in each soil treatment was compared in an infectivity assay.

The soil samples from Auscott, Dalby and Warren were collected from within a crop of irrigated cotton, before harvest, during late summer, 1994, and from Auscott again in 1995 and 1996. The top 5 cm of soil was removed and tubes 5 cm diameter and 15 cm long were then pressed vertically into the soil as far as the weight of the collector permitted. The tubes, in which the soil was 12–15 cm deep, were returned to the laboratory and stored dry.

Bulk soil from the Research Institute was collected and also stored dry. In experiments, the soil was broken apart, sieved through a 5-mm mesh and then mixed three parts to seven parts coarse sand, by weight. The soil mixture was autoclaved for 90 min in shallow trays and stored dry.

Influence of disturbance on infectivity of soil

Rates of root infection in undisturbed and disturbed soil were compared. Tubes of Waverly soil were either left as blocks or were disturbed severely

Table 1. *Seedlings per tube and length of growth period of* Trifolium subterraneum *planted in disturbed and undisturbed blocks of soil*

Harvest, days after transplanting	4	6	10	15	21	28	42
Number of seedlings harvested per tube	10	9	8	7	6	4	2

immediately after collection, then saturated by placing the tubes in a water bath for 48 h. After 12 h draining, during which disturbed soil packed down, each tube was weighed and the weight recorded on the tube. Germinated seedlings of *Trifolium subterraneum* L. (Seaton Park) with roots *c*. 1 cm long were transplanted to each tube (Table 1). Plants were grown in a growth room at an irradiance of 650 μmol m^{-2} s^{-1} with day length of 13 h at 25 °C and night temperature of 20 °C, and watered to weight every 2 d with tap water. More seedlings were transplanted to tubes designated for early harvests to increase root exploration of these soils. Tubes were harvested at 4, 6, 10, 15, 21, 28 and 42 d after transplanting, roots of all plants from eight replicate tubes cleared, stained in trypan blue (Phillips & Hayman, 1970) and the extent of AM determined by the grid-intersect method (Giovannetti & Mosse, 1980).

Propagules that initiate infection

The rates at which seedlings became mycorrhizal from inocula of spores, root fragments or untreated, undisturbed soil were compared. Spores and root pieces were extracted from blocks of 56 $\pm$ 2 g soil from Waverly following dispersal in 1% solution (w/v) of Na_3PO_4, a concentration found to have no effect on fungal viability (P. A. McGee, unpublished). Potential propagules remaining on a 56-μm grid were rinsed three times in sterile deionized water and stored for no more than 24 h in water at 4 °C. Inocula of either spores from one block, root pieces from one block, or one untreated block were placed *c*. 3 cm below the surface of the soil in the centre of closed pots containing 750 g autoclaved soil/sand mix at 75% field capacity to maintain some soil aeration. An uninoculated control was included. Three germinated seeds of *T. subterraneum* were placed above the inoculum. Six replicate pots of each treatment were harvested after 10 or 20 d, and the roots were extracted, cleared and stained, and their length and that of mycorrhizal roots measured microscopically.

Further fragments of senescent root were extracted from blocks of soil and placed in dilute H_2O_2 until clear. The cleared fragments were then stained in trypan blue and examined microscopically for mycorrhizal fungi.

Spore counts

Spores were extracted from 10 g samples of dry soil from freshly collected cores from Auscott, Warren, and Dalby in 1994. Three cores of soil from each location were each sampled twice. The spores were wet-sieved and extracted by centrifugation in 48% sucrose (Daniels & Skipper, 1982). All spores apparently filled with lipid materials were counted. Obviously empty, broken or flattened spores were ignored.

Further spores from the dried field soil from Auscott were extracted and identified to family level. The aim was to determine whether isolates that might have dormancy were present in the Auscott soil collected in 1994. We were only interested in whether or not the fungi were members of the Acaulosporaceae. The only criterion that was used was presence or absence of a long subtending hypha. The undescribed species of *Acaulospora* we have previously isolated from the Auscott and other soils, which we have in pot culture, is readily distinguished because of a short attachment to the endospore, *c*. 5–10 μm long. Spores were extracted from four 25 g samples of dry soil.

Viability of spores

In the remaining experiments, plants were grown in a growth room with 12-h day at 25 °C at an irradiance of *c*. 1000 μmol m^{-2} s^{-1} and night temperature of 18 °C. The soil/sand mix was watered every 2 d with deionized water.

Because spores were present in field soil at high densities, their potential to initiate infection was tested. In the first experiment, spores in soil cores collected from Auscott in 1994 that had been stored dry for 1 yr were compared with spores from freshly collected soil . Spores were extracted from soil, and collected on 100-μm and 50-μm sieves because of the suggestion that large spores are more likely to be viable than small spores (Brundrett & Juniper, 1995). For comparison, spores were also extracted from a fresh pot culture of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe (NBR 4.1), a fungus isolated from soil at Auscott. From each grid, all spores were removed, placed in sterile water and stored in water at 4 °C for up to 24 h until used. Approximately 5, 10 or 20 lipid-filled spores from each sieve were then placed at the bottom of a funnel-shaped piece of Parafilm® placed 5 cm below the soil surface in pots of 570 g moistened sterile soil/sand mix. Ten seeds of leek, *Allium porrum* L. were placed on the soil surface above the spores and the pots were placed in a growth room. Seedlings were thinned to four after germination. Ten replicate pots were harvested after 6 wk. All the roots of seedlings were cleared and stained for AM, and each pot scored for presence or absence of AM.

The experiment was repeated using soil collected from Auscott in 1994 and stored for 2 yr, and soil collected in 1996. On this occasion, some spores were used in the bioassay and others were stained with vital stains. Ten replicate pots of 20 spores extracted on 50-μm and 100-μm sieves were harvested after 6 wk and examined for the presence of AM.

To determine the proportion of potentially viable spores, *c.* 20 spores suspended in distilled water were mixed with an equal volume of staining solution to reach a final staining concentration of 1 mg ml^{-1} of 2,3,5-triphenyltetrazolium chloride (tetrazolium red; Walley & Germida, 1995) in an unbuffered 0·1 M solution of succinic acid (Meier & Charvat, 1993; Saito, Stribley & Hepper, 1993) in microtitre wells. After 72 h incubation in the dark at 25 °C, six replicate wells per treatment were assessed for colouration.

All mycorrhizal roots of plants grown in stored and in freshly collected field soil were examined microscopically to identify the AM fungi that initiated infection, using the keys of Abbott & Robson (1978) and McGee (1989). Identity of mycorrhizal fungi was confirmed by comparing the unknown fungi with mycorrhizas established from an unidentified species of *Acaulospora* isolated to pot culture from soil at Auscott, and isolates of *Glomus intraradices* Schenck & Smith and NBR 4.1, also from Auscott.

Survival of hyphae

Every 4 wk for 32 wk four cores of soil collected from Narrabri in 1995 were examined for viable hyphae using the method of Sukarno, Smith & Scott (1993). A 3-g subsample was taken from each core and placed in 200 ml of 2% Calgon® solution, mixed for 5 min and four 2-ml aliquots taken and passed through a 8-μm nitrocellulose filter (Millipore). A 1-ml solution of 5 μM fluorescein diacetate (FDA) was placed on the surface of the retained material. After storage in the dark for 20 min, the stain was removed and each filter placed on a microscope slide with 50% glycerol to reduce drying. Each filter was examined under a Zeiss fluorescence microscope with excitation filter BP450–490 and barrier filter LP 520. The length of stained hyphae was determined using the grid intersect method at 100 × magnification on 20 fields of view, and length was expressed according to the d. wt of the soil.

RESULTS

Influence of disturbance on infectivity of soil

Plants grown in the undisturbed soil became mycorrhizal within 4 d, and the proportion of the roots that were mycorrhizal increased rapidly until *c.* 45% were infected after 42 d (Fig. 1). Infection was both delayed and reduced in the sieved soil. The proportion of roots infected by mycorrhizal fungi was not significantly different from zero until after 15 d, and at 42 d was *c.* 30% (Fig. 1). The length of roots at each harvest were statistically similar in both treatments.

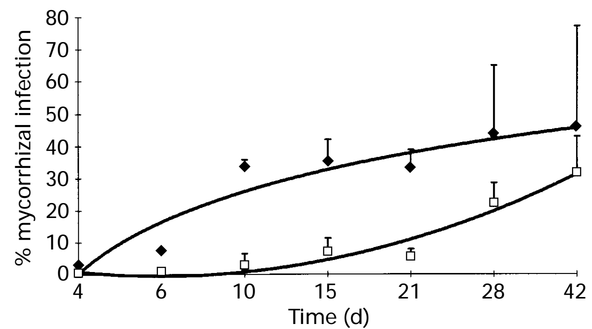

Figure 1. Mean (±SE) proportion of mycorrhizal roots in trap plants grown in cores of disturbed (□) and undisturbed soil (◆).

Table 2. *Mean number of spores (range) of AM fungi in soils from Auscott, Narrabri, Dalby and Warren*

Soil source	Mean number spores g^{-1} soil (range)	Proportion of genera of AM present
Auscott	20·7 (8·7–39)	34% *Acaulospora* 66% *Glomus*
Dalby	75 (4–212)	n.d.
Warren	14·6 (3·6–34)	n.d.

n.d., not determined.

Propagules that initiate infection

A mean (±SD of 225±23 spores (mean 4 g^{-1} soil) and 0.2±0.1 g dry root fragments were extracted from the Waverley soil. Plants inoculated with root pieces had no infection at 10 or 20 d. Plants inoculated with spores were uninfected at 10 d and had 3±1 cm mycorrhizal roots at 20 d. Plants inoculated with blocks of soil had 4±1 cm mycorrhizal roots at 10 d and 18±5 cm at 20 d (*c.* 10% root length infected at both harvests). The length of roots of trap plants in each treatment were not significantly different at each harvest.

Fungal hyphae were uncommon in the stained root fragments, some of which had a complex of stained hyphae over the root surface. Most of the hyphae lacked cytoplasm and appeared to be dead. No arbuscules, vesicles or spores were seen.

Spore counts

Spore density ranged from 3·6 to 212 spores g^{-1} (Table 2). No effort was made to identify all spore types, but soils with higher densities contained

Table 3. *Percent of pots in which AM were found in the roots of* Allium porrum *after 6 wk using inoculum of 5, 10 or 20 spores, and mean percentage of spores stained positively with tetrazolium red*

Source of spores and diameter of spores	Inoculum …	Expt 1: % pots found with AM (% spores) 5*	10	20	Expt 2: % pots with AM (% spores) 20	Mean % stained spores 20
Freshly collected soil						
50–100 μm		0	10 A (1)†	10 A (0·5)	30 A, G U (1·5)	21 X
> 100 μm		10 G (2)	60 G, U (6)	20 G (1)	20 G, (1)	16 A, X, Y
Stored dry in soil for 12 months						
50–100 μm		10 A (2)	20 G, A (2)	40 G, A, U (2)	–	–
> 100 μm		0	0	0	–	–
Stored dry in soil for 24 months						
50–100 μm		–	–	–	0	7 X, Y
> 100 μm		–	–	–	20 G (1)	6 Y
Pot culture of NBR 4·1						
50–100 μm		0	10	10	40	8 X, Y
> 100 μm		20	60	70	90	7 Y

* No. of spores.
† Percentage of infective spores shown in parentheses assuming each infection is initiated by a single spore. Numbers followed by A were found to be infected with *Acaulospora*, G with *Glomus* and U by an unknown fungus; by X alone differ significantly ($P = 0{\cdot}05$) from those followed by Y alone. Both X and Y are similar when numbers are followed by XY.

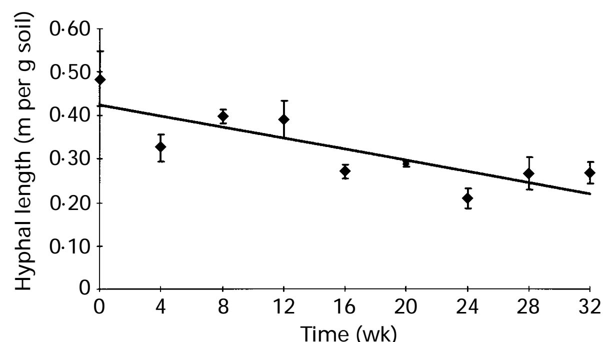

Figure 2. Mean (±SD) length (m) of fluorescing hyphae extracted over 32 wk from soil stored dry.

more spores that were tentatively identified as *G. intraradices*. A total of 312 identifiable spores were extracted from the four soil samples from Auscot; 106 were tentatively identified as *Acaulospora* and the remainder assumed to be *Glomus* (Table 2). No members of the Gigasporaceae have been found in soils used to grow cotton. Roots were absent in most soil samples.

Viability of spores

All roots of the trap plants passed through the base of the funnels. In the first and second bioassay, few spores from freshly collected soil and even fewer from stored soil initiated AM (Table 3). The morphology of arbuscular mycorrhizal fungi was examined in all roots. In the first bioassay, *Glomus* was found in five pots, *Acaulospora* in two and unknown fungi in four inoculated with fungi from freshly collected field soil. In field soil stored for 12 months, the fungi in five root systems resembled *Acaulospora* and in one, *Glomus*; one root system contained infections of both *Acaulospora* and *Glomus* and two could not be identified (Table 3). All infection units were very short.

In the second bioassay (Table 3), *Glomus*, *Acaulospora* and unidentified fungi were present in plants grown in the freshly collected soil. Where the soil had been stored for 24 months, *Glomus* but not *Acaulospora* was found in both pots. Again, the infection units were short.

More spores from the pot culture initiated AM in both experiments (Table 3) and secondary spread was evident in these roots.

Spores that were pink or red after 72 h were counted as positively stained (Table 3). No black or brown spores were observed. In general, smaller spores stained within 24 h, larger spores stained after 48 h, and those with hyphal sheaths did not stain (Meier & Charvat, 1993).

Survival of hyphae

The length of hyphae that fluoresced declined significantly from 0·5 m g^{-1} to 0·3 m g^{-1} of soil over

the 32-wk period (Fig. 2). A regression line was fitted to the data with the equation $y = 0{\cdot}450–0{\cdot}023x$, with an r^2 value of 0·647.

DISCUSSION

In this investigation, we found that in both cultivated and uncultivated soils spores were common, senescent roots were uncommon and the hyphal network was probably an important source of infection by arbuscular mycorrhizal fungi.

In the uncultivated soil from Waverley, the imposition of a very severe disturbance reduced the rate at which AM formed in the roots of trap plants. We assumed from this that the reduction was the result of hyphae being shattered by crushing and sieving. Our assumption was supported by the negligible infection arising from spores and root pieces extracted from blocks of undisturbed soil. We did not succeed in extracting hyphae from soil satisfactorily on this occasion and are unaware of the length of hyphae and their viability in the soil from Waverley. We have observed a similar decline in rates of infection in sieved soil from a vegetation reserve near the Research Institute (McGee & Masuhara, unpublished). The rate of infection might have been influenced by differing moisture conditions. However, as the sieved soil packed down on wetting, and root growth was similar in disturbed and undisturbed soil, soil water was unlikely to be a significant factor. These data support the suggestion that in undisturbed soil AM are commonly initiated from the hyphal network in soil, as is observed in sandy soils of Australia (southern WA, (Jasper, Abbott & Robson, 1991); SA, (McGee, 1989); the Hawkesbury plateau, NSW, (Bellgard, 1993); the Sydney Basin (G. S. Pattinson & P. A. McGee, unpublished)).

In the cracking heavy clay soils, the fungus might, in addition, regenerate from spores. The proportion of fresh spores able to initiate AM appears to be low. If the experimental conditions were conducive to spore germination, the low level is possibly due to two factors, dormancy in some species and the presence of a large proportion of unviable spores.

The spores of some members of Acaulosporaceae have periods of innate dormancy (Tommerup, 1983*a*; Gazey *et al.*, 1993). Few infections of the *Acaulospora* type were observed, and therefore dormancy is unlikely to be the cause of low rates of infection. Further, although spores of Acaulosporaceae are common, they appear unable to survive long periods in dry soil.

A small proportion of the spores found in field soil were found to be infective in the bioassay. We do not know how many spores are needed to initiate a maximum level of mycorrhiza in field plants. If we assume: (i) the root length of a single plant is 5 m kg^{-1} soil at 6 wk (unpublished data from the experiment on disturbance and other work with *T. subterraneum*); (ii) the maximum level of mycorrhizal infection is 50 % (data from Fig. 1); (iii) a mean of 0·5 cm mycorrhizal root forms per infective unit (conservative estimate); then a minimum of 5 spores g^{-1} soil would be required to initiate maximum levels of infection. A minimum of 6 % of the 21 spores extracted g^{-1} freshly collected soil from Auscott, 2 % after 12 months and 1 % after 24 months storage, were infective. This suggests that in the soil from Auscott, spores contribute a small proportion of the initial infection and the infection is probably inititated by fragments of hyphae in the soil in most cases. Moreover, as soil ages in the absence of host plants, hyphae assume an increasing importance as infective units.

Because of this conclusion, it is necessary to consider the assumptions carefully. For instance, the experimental design might have reduced the number of spores which initiated infection. Roots of the trap plant passed through the spores at the base of the funnel, and potentially infectable roots would have been present for short periods. If the spores had been distributed randomly, a larger number of infection units might have been initiated (Abbott & Robson, 1984), indicating more viable spores. If we accept that the design was appropriate, the data indicate that viability of spores is initially low and declines over time.

We examined a range of vital stains in this investigation (and unpublished), with the aim of finding a fast method of determining viability of field-collected spores. Of the stains we examined, only tetrazolium red gave consistent results in consecutive experiments, but the results were dissimilar to data from bioassays. In the data we presented, the possible reasons for the dissimilarity include the lack of penetration of stains into mature spores and the possible staining of cytoplasm in immature spores which do not germinate and initiate AM. The dissimilarity of data from bioassays and from vital staining in spores from pot culture and the field leads us to suggest that all data on staining spores with vital stains needs careful interpretation.

The changes in the viability of spores found by bioassay also lead us to suggest that size of the spore is a poor guide to its potential survival in field soils. Approximately five of 55 spores of less than 100 μm diameter compared with 11 of 55 spores over 100 μm diameter from freshly collected soil initiated infection. At 12 months all spores that established infection were smaller than 100 μm, and at 24 months all spores were larger than 100 μm, indicating that survival of spores is unrelated to size.

Maturity of spores might be important to spore survival. In pot culture, the mature spores of NBR 4.1 are larger than 100 μm in diameter and more of

the larger spores initiated AM. The data also indicate the difficulty of using data from spores grown in pot culture to predict results under field conditions.

We examined length of survival of hyphae in soil by vital staining. We were unable to develop a bioassay to support the results and so have limited confidence in the conclusion. If vital staining indicates the potential capacity to initiate infection by hyphae then the survival of hyphae declines linearly with age.

Our observations have implications for our understanding of the initiation of AM, especially following long fallows. Firstly, time seems to be an important component determining the survival of both hyphal fragments and spores in field soil. The infectivity of arbuscular mycorrhizal fungi appears to decline over time in the absence of any direct action on the fungal propagules. Secondly, cultivation possibly contributes to the decline in density of arbuscular mycorrhizal fungi in soils used to grow cotton. Although we imposed an especially severe disturbance in this study, our data are consistent with results on disturbance published elsewhere (Jasper *et al*., 1987, 1991; Evans & Miller, 1990). Thirdly, long fallows usually include falls of rain, potentially leading to germination of non-dormant propagules. In the absence of a host, the propagules die if they cannot re-establish quiescence (Braunberger, Abbott & Robson, 1994). Thus, densities of arbuscular mycorrhizal fungi are likely quickly to reach critically low densities for adequate plant growth, as observed by Thompson (1987).

Fungi found in the clay soils in eastern Australia used to grow cotton share some features with those from cultivated soils of southern Australia. Spores are present in significant densities in cultivated soils and they appear to be important for initiation of AM. Further, the hyphal network, which also contributes significantly to initiation of AM, might be damaged during cultivation. In contrast, fungi in senescent roots (Tommerup & Abbott, 1981) apparently initiate few AM in soils used to grow cotton.

This research indicates that, in the absence of a host plant, the density of arbuscular mycorrhizal fungi declines in cultivated clay soils used to grow cotton. The rate of decline and the relative ability of each component of the fungus to initiate AM remains unquantified. The decline might be exacerbated by cultivation. Such a conclusion suggests that 'long fallow disorder' should be a major problem in soils used to grow cotton, but is uncommon in cotton, a plant dependent on mycorrhizas. This means either that a quantity of propagules of arbuscular mycorrhizal fungi exists for greater than that required to establish adequate densities of AM in cotton or that methods used to quantify the surviving fungi do not reflect their relationship to initiation of AM in that host, or that cultural practices have only a minor effect on the fungi.

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