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Mycorrhiza

The topsoil as the major store of the propagules of vesicular-arbuscular mycorrhizal fungi in southeast Australian sandstone soils

S. E. Bellgard*

Department of Biology, University of Wollongong, Wollongong, NSW 2500, Australia

Abstract. The formation of vesicular-arbuscular mycorrhizae (VAM) in intact soil profiles from two sites in southeastern Australia were measured at two depths using a bioassay grown in intact soil cores. Intact soil cores were taken from (1) topsoil (0–15 cm) and (2) subsoil (15–30 cm) four times during 1990. Seeds of *Acacia linifolia* (Vent.) Willd. (Mimosaceae) were sown into the cores and plants harvested 8 and 12 weeks after sowing. For 1990, at both sites and in all seasons, VAM most readily developed in the roots of seedlings of *A. linifolia* grown in topsoil. Limited VAM occurred in roots grown in subsoil cores. Most colonisation of roots by VAM occurred from cores collected during spring and summer. Spore numbers were quantified for each site and depth by wet-sieving 100-g samples of air-dried soil and counting turgid spores containing oil droplets. Three types of spores were found in the soils. Few spores were extracted from all soils sampled, and for the most abundant of the spore types at least twice as many spores occurred in the topsoil than in the subsoil for all seasons examined. As most of the propagules that initiate VAM infection were observed in the topsoil, disturbances which involve the removal and storage of the top 15 cm will adversely affected these fungi.

Key words: Vesicular-arbuscular mycorrhizae fungi – Propagules – Spores – Topsoil – Sandstone soils

Introduction

The sandstone soils surrounding the Sydney region in southeastern Australia are characterised by their poor structure and low fertility (Beadle 1954, 1962). Soils with poor structure, low to moderate clay content, and low levels of essential plant nutrients (especically phosphorus) are typical of a range of ecosystems, e.g. arid (White et al. 1989), semi-aried (McGee 1986), and forest soils of southwestern Australia (Jasper et al. 1989a). Several studies have shown that vesicular-arbuscular mycorrhizal (VAM) infection can significantly improve the phosphorus nutrition and yield of plants growing in soils of low fertility (Mosse 1973; Harley and Smith 1983). Bradshaw (1983) proposed that the primary stress imposed on seedlings recolonising disturbed areas is a lack of nutrients. Disturbance of soil (as a result of agriculture and mining practices) has been shown to reduce the infectivity of VAM fungi (e.g. Moorman and Reeves 1979; Parke et al. 1983; Fairchild and Miller 1988; Jasper et al. 1989b, c). Consequently, it has been hypothesised that if viable VAM inoculum is absent or reduced in post-disturbance situations, then the development of the recolonising plant community may be limited or retarded, particularly in soils that have low concentrations of essential plant nutrients (Loree and Williams 1987).

Disturbance normally involves a mixing of the horizons of the soil profile. Consequently, the vertical distribution of VAM fungi in the soil profile will determine in part the degree to which the VAM fungi are affected by the disturbance. This may in turn influence the amount of viable VAM inoculum available to initiate infection in the recolonising plant species (Schwab and Reeves 1981). Numerous studies have shown that the upper soil profile contains the greatest number of fungal spores, and that both the number of spores of each species and the number of species decreases with increasing depth (e.g. Smith 1978; Mertz et al. 1979; Koide and Mooney 1987). However, some species of VAM fungi may not even produce spores (e.g. Johnson 1977; McGee 1989). Consequently, little is known about the distribution of VAM fungi in the soil profile despite their demonstrated importance in plant establishment and growth. While data on the distribution of VAM fungi have been obtained using spore counts (e.g. Porter 1979; Gemme and Koske 1988), infected root fragments (e.g. Powell 1976), hyphal tips (e.g. Buchholtz and Motto 1981), and MPN (McGee 1989), these techniques do not measure the potential of soil to initiate VAM infection (e.g. Moorman and Reeves 1979; Rives et al. 1980). Since the most im-

* *Present address and address for correspondence:* Science and Information Division, Department of Conservation and Land Management, 50 Hayman Road, Como, WA 6152, Australia

portant propagules of VAM fungi in soils are generally unknown or not determined (Brundrett 1991), a bioassay is considered to give a measure of the total infectivity of these fungi (Schwab and Reeves 1981).

Soil disturbance in the plant communities under investigation usually involves the removal and storage of the top 15 cm of the soil profile. While studies investigating the impacts of topsoil removal and storage on the infectivity of VAM fungi have been carried out in Western Australia (e.g. Jasper et al. 1988, 1989a, b, c), no such studies have been attempted in southeastern Australia. Before investigating the impacts of soil disturbance on VAM fungi, it was necessary to identify where in the intact soil profile VAM fungi were most active. This study aimed to: (1) identify the section of the soil profile in which VAM fungi are most infective, and (2) investigate the relative importance of spores as a potential source of VAM infection.

Materials and methods

Site description

The southern part of the Hawkesbury Sandstone plateau, located 90 km south of Sydney and to the west of Wollongong, Australia, covers approximately 1200 km^2. The average annual precipitation in this region is 1420 mm, with a slight summer predominance in distribution. The two study sites examined were located within the catchments of the Avon (34°22′S, 150°40′E) and O'Hares (34°14′S, 150°53′E) dams. The vegetation of the Avon site is a woodland with an overstorey of *Eucalyptus haemostoma* Sm. and *E. racemosa* Cav. (Myrtaceae) and a shrub understorey comprising a variety of genera including *Acacia, Banksia, Grevillea, Isopogon,* and *Lomandra.* The O'Hares site supported a sclerophyllous open heath dominated by *Angophora hispida* (Sm.) Blaxell (Myrtaceae) associated with species of *Acacia, Leptospermum, Banksia, Conospermum, Grevillea,* and *Persoonia.*

Physical and chemical analysis of soils

Soil samples were taken from five randomly chosen locations at each site. Soil samples were taken from 0–15 cm and 15–30 cm, placed in plastic bags and transported back to the laboratory. The soil was passed through a 2-mm sieve, the pH was measured, and the soil air-dried. The following physical and chemical analyses were completed on all soil samples by the Sydney Water Board: (1) pH using a water to fresh soil ratio of 2:1; (2) soil particle size distribution using a Bouycos hygrometer (Ball 1986); (3) loss on ignition (Allen et al. 1986); (4) total phosphorus using a perchloric acid digestion (Hesse 1971); (5) bicarbonate-extractable phosphorus (Colwell 1965); (6) total nitrogen using micro-Kjeldahl digestion (Hesse 1971); and (7) soluble sodium, calcium, and magnesium using a 1-M ammonium acetate extraction (pH 7.0) at a soil to extract ratio of 1:25 (Allen et al. 1974).

Procedure of the VAM bioassay

At the start of each season (March 1990, autumn; June 1990, winter; September 1990, spring; December 1990, summer), intact soil cores were removed from 16 locations chosen at random within each site. A topsoil (0–15 cm) and a subsoil (15–30 cm) core were sequentially removed and placed on gravel in a 20-cm plastic pot to prevent soil washing through.

Table 1. Daily temperature ranges (°C) in the glasshouse for each season

Season	Minimum (mean ± SEM)	Maximum (mean ± SEM)
Autumn	14.9 ± 1.8	23.5 ± 1.4
Winter	12.6 ± 1.3	19.3 ± 0.9
Spring	16.6 ± 1.8	25.3 ± 2.2
Summer	21.0 ± 2.0	30.7 ± 3.3

Seeds of *Acacia linifolia* (Vent.) Willd. (Mimosaceae), a local plant known to form VAM associations (Bellgard 1991) were immersed in boiling water and left until the water had cooled to increase their germinability (Cavanagh 1987). The seeds were germinated in bubbling, sterile, deionised water prior to sowing (Jasper et al. 1987). Fifteen pre-germinated seeds were sown into each of the pots. All pots were watered twice daily with tap water, and no additional nutrients were applied. The were placed in a naturally lit, outdoor glasshouse for the duration of the bioassay (Table 1). To monitor for aerial contamination of VAM fungi in the glasshouse, 16 pots of river sand were sown with 15 pre-germinated seeds of *A. linifolia.*

Eight and 12 weeks after sowing, five randomly chosen seedlings were harvested from each of the 16 topsoil, subsoil and control pots. Each seedling was carefully extracted from its medium and its roots were washed gently in a 0.4% solution of sodium hexametaphosphate, which dispersed any adhering soil. The roots of each seedling were fixed in 50% ethanol solution, cleared and stained (Kormanick et al. 1980). Total root length and the portion of root colonised by VAM fungi were determined (Ambler and Young 1977). The length of root colonised by VAM fungi (VAM length) is a composite index, the sum of the lengths of root colonised by vesicles, arbuscules and internal hyphae (NB. % VAM = VAM length/root length × 100).

Sampling and extraction of spores

Soil samples from 0–15 cm and 15–30 cm were collected at a further 25 randomly chosen locations within each of site at the start of each season. Spores of VAM fungi were obtained by wet-sieving and decanting 100-g subsamples of air-dried soil. Turgid spores filled with oil droplets were considered viable (McGee 1989) and counted microscopically.

Statistical analysis

The VAM formation data were analysed using a three factor "split-plot" analysis of variance (Cochran and Cox 1957) with depths nested within cores, and cores nested within sites. The treatment structure was a "season × depth × time" factorial structure, with the factor "season" having four levels (i.e. autumn, winter, spring and summer), the factor "depth" having two levels (i.e. topsoil = 0–15 cm and subsoil = 15–30 cm), and the factor "time" having two levels (i.e. 8 weeks and 12 weeks).

The analyses of the density of VAM spores were carried out using a two factor "split-plot" analysis of variance with depths nested within cores, which were nested within sites. The treatment structure was a "season × depth" factorial with the factor "season" having four levels (i.e. autumn, winter, spring and summer), and the factor "depth" having two levels (i.e. topsoil = 0–15 cm and subsoil = 15–30 cm).

Table 2. Physical-chemical analysis of soils from the study sites. Entries given are means ± SEM (n = 5). Total P, total phosphorus; Extr P, bicarbonate-extractable phosphorus; Total N, total nitrogen; Sol. Na, Ca, Mg, soluble sodium, calcium, magnesium, respectively

	Avon				O'Hares			
	0.15 cm		15–30 cm		0–15 cm		15–30 cm	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
pH	5.6	0.1	5.7	0.2	5.3	0.1	5.3	0.2
Gravel (%)	12.6	2.5	9.6	2.2	32.3	3.8	42.0	10.6
Sand (%)	61.7	2.0	56.9	3.3	55.0	11.3	42.0	9.4
Silt (%)	15.6	2.0	20.8	1.4	6.7	0.7	8.4	0.7
Clay (%)	10.1	0.5	12.7	0.8	6.0	0.5	7.6	0.7
Organic matter (%)	10.4	2.1	8.8	0.6	3.1	0.8	2.7	1.2
Total P (ppm)	30.0	3.2	17.0	3.8	13.0	3.0	14.0	2.5
Extr P (ppm)	0.7	0.1	0.5	0.0	0.8	0.3	1.2	0.5
Total N (ppm)	786.0	83.2	512.0	88.3	468.0	131.5	106.0	6.3
Sol. Na (ppm)	91.0	4.4	92.0	1.2	106.0	15.7	136.0	16.3
Sol. Ca (ppm)	204.0	17.5	92.0	1.2	160.0	15.7	136.0	16.3
Sol. Mg (ppm)	139.2	19.9	110.4	22.3	112.8	6.2	151.2	15.7

Results

Soils

The soil at the Avon site had 30 cm of yellow clayey subsoil overlain by 20 cm of loamy sand topsoil [Yellow Earths (Gn2.21)], while the soil at O'Hares was a loose, shallow sand of 30 cm with abundant ironstone overlying the sandstone surface [Lateritic-Podzolic Soils (Dy 3.61)] (Hazelton and Tille 1990). Physical and chemical properties of the two soils differed especially with respect to texture, organic matter content, and the concentration of some important nutrient elements (Table 2). Little difference in the physical or chemical composition was found between the topsoil and the subsoil at either site (Table 2).

Root growth and formation of VAM associations

No VAM fungi were found on the roots of the bioassay seedings grown in the control pots. Roots were similar length in topsoil and subsoil. The trend observed in the length of root colonised by VAM fungi (i.e. VAM length) and the proportion of root length colonised by VAM fungi (i.e. %VAM) were identical. Consequently, only the proportion data are presented. The development of VAM infection was greater in the roots of plants grown in topsoil than subsoil at both sites, at both sampling dates, and for all seasons (Fig. 1; Table 3). Limited VAM formed in roots of plants grown in the subsoil cores taken from both sites (Fig. 1; Table 3). More VAM associations formed in the soil cores sampled and assayed during spring and summer than during autumn and winter in both sites and at both soil depths (Fig. 1; Table 3).

Spore types and abundance

Three types of spores of VAM fungi were found in the soils of both sites, but spore numbers were generally low. Type I spores were globose, hyaline, 160 µm wide with a membranous wall and no hyphal attachment. Type II spores were roughly oblong in shape, brown, 190 µm wide, with an ornamented wall and no hyphal attachment. Type III spores were spherical, amber, 90–120 µm wide, with a thin membranous wall and no hyphal attachment.

Generally, at both sites and in all seasons, more spores were found in the topsoil than in the subsoil. Type I spores were the most abundant and these spores were significantly more numerous in topsoil than in subsoil at both site sand for each season examined (Fig. 2; Table 4). Few type II spores occurred and they were evenly distributed through the soil during autumn and summer, but significantly higher numbers were found in the topsoil than in the subsoil during spring and winter (Fig. 2; Table 4). Type III spores were uncommon (Table 4).

Discussion

VAM associations formed more readily in seedlings grown in topsoil than in subsoil. The difference in VAM formation may be due to differences in the physical/chemical properties of these two soil horizons. Soil moisture, temperature (Daniels and Trappe 1980; Parke et al. 1983; Abbott and Robson 1991), soil pH (Black and Tinker 1979), and the concentration of inorganic nutrients (Slankis 1974) have been shown to influence the growth and development of VAM fungi. However, few differences were observed between the physical/chemical properties of the topsoil and subsoil at each of the sites. Consequently, the differences in VAM formation between topsoil and subsoil indicate that most of the propagules that initiate VAM infection in these soils

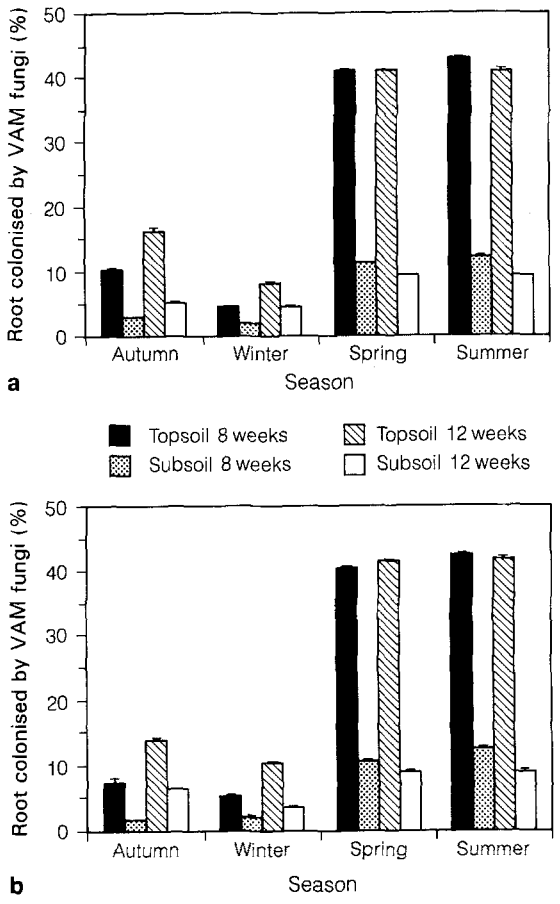

Fig. 1. Proportion of root length colonised by VAM fungi in 8- and 12-week-old seedlings growing in intact soil cores taken from two soil depths (topsoil = 0–15 cm and subsoil = 15–30 cm) from **a** the Avon and **b** the O'Hares study sites in southeastern Australia. Bars represent the means (± SEM) of 16 replicates

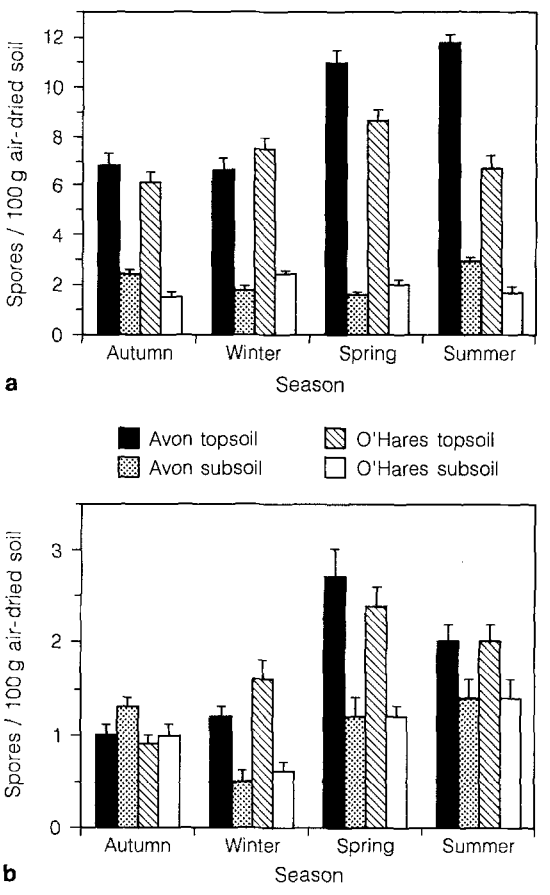

Fig. 2. Density of **a** type I and **b** type II spores at two soil depths (topsoil = 0–15 cm, subsoil = 15–30 cm) from two sites in southeastern Australia. *Columns* represent mean numbers of fungal spores (± SEM) for 25 random samples taken at the start of each season

Table 3. Details of the analysis carried out on % VAM data from the Avon and O'Hares sites using a three-factor "split-plot" ANOVA with depths nested within cores, which are nested within sites. Significant synergistic interactions occurred in all cells. *A posteriori* comparisons were carried out using the appropriate LSD's to identify differences between means. Data given are means for five samples taken from each of 16 replicates harvested 8 and 12 weeks after sowing in each of four seasons. Entries in vertical columns followed by the same letter are not significantly different as indicated by analysis of variance (LSD = 1.1, $P = 0.01$). Entries in horizontal rows followed by the same superscripted number are not significantly different as indicated by analysis of variance (LSD = 1.2, $P = 0.01$). Entries in italics are significantly different from the corresponding season × depth entries at 8 weeks as indicated by analysis of variance (LSD = 0.9, $P = 0.01$)

	Autumn	Winter	Spring	Summer
8 weeks				
0–15 cm	8.8a[1]	4.8a[2]	40.7a[3]	42.6a[4]
15–30 cm	2.2b[1]	1.9b[1]	10.9b[2]	12.5b[3]
12 weeks				
0–15 cm	*14.9a*[1]	*9.2a*[2]	*41.6a*[3]	*41.4a*[3]
15–30 cm	*5.8b*[1]	*4.1b*[2]	*9.1b*[3]	*9.3b*[3]

occur in the top 15 cm of soil. However, only 11 soil parameters were examined in this study (Table 2). It many be that some unmeasured factor e.g. toxic levels of metals (Guildon and Tinker 1983; Koslowsky and Boerner 1989) or dormancy of spores (e.g. Tommerup 1983a) prevented the development and spread of infection of VAM in the subsoil cores.

The highest amount of VAM formation occurred during spring and summer. It is generally accepted that the optimal temperature range for VAM growth is between 18 and 24 °C (e.g. Slankis 1974; Daniels and Trappe 1980; Parke et al. 1983). In this experiment, the daily range of glasshouse temperatures during spring and summer coincided with the quoted optimal range for VAM formation, and this may explain why VAM formation peaked during these seasons. During autumn and winter, glasshouse temperatures ranged between 12.6 and 23.5 °C. Smith and Bowen (1979) observed that the onset of VAM infection was delayed at low temperatures, suggesting that VAM fungal activity is inhibited at low temperatures. Tommerup (1983b) demonstrated that the average rate of hyphal extension of selected species of VAM fungi decreased at low temperatures. In addition, differences occur between VAM fungi in the op-

Table 4. Details of the analysis carried out on the density of VAM spores of each type from the Avon and O'Hares sites using a two-factor "split-plot" ANOVA with depths nested within cores, which are nested within sites. Entries given are mean number of fungal spores. Twenty five random samples were taken at the start at each of the seasons

	Autumn	Winter	Spring	Summer
Type I[a]				
0–15 cm	6.46a[1]	7.02a[2]	9.72a[3]	9.20a[3]
15–30 cm	1.96b[1]	2.08b[1]	1.80b[1]	2.28b[1]
Type II[b]				
0–15 cm	1.00a[1]	1.24a[1]	2.54a[2]	1.98a[2]
15–30 cm	1.40a[1]	0.90a[2]	0.96b[2]	1.36b[1]
Type III[c]				
0–15 cm	0.04a[1]	0.08a[1]	0.14a[1]	0.12a[1]
15–30 cm	0.00a[1]	0.00a[1]	0.10a[1]	0.10a[1]

[a] Entries in vertical columns followed by the same letter are not significantly different as indicated by analysis of variance (LSD = 1.02, $P = 0.01$). Entries in horizontal rows followed by the same superscripted number are not significantly different as indicated by analysis of variance (LSD = 1.04, $P = 0.01$)

[b] Entries in vertical columns followed by the same letter are not significantly different as indicated by analysis of variance (LSD = 0.42, $P = 0.01$). Entries in horizontal rows followed by the same superscripted number are not significantly different as indicated by analysis of variance (LSD = 0.43, $P = 0.01$)

[c] Entries in vertical columns followed by the same letter are not significantly different as indicated by analysis of variance (LSD = 0.13, $P = 0.01$). Entries in horizontal rows followed by the same superscripted number are not significantly different as indicated by analysis of variance (LSD = 0.12, $P = 0.01$)

timal and lower temperature limits for spore germination (e.g. Schenck et al. 1975; Tommerup 1983a, b; Daniels and Trappe 1980). In the current study, there appears to be a pattern in the infectivity of VAM fungi related to seasonal temperatures, with higher infectivity being associated with the higher temperatures of spring and summer.

The relative importance of spores, old roots, and soil hyphae as propagules apparently varies between species of VAM fungi occurring in the same site (McGee 1989). Since the most important propagules of VAM fungi in Hawkesbury Sandstone soils were unknown, the bioassay of intact soil cores described above was used to measure the total infectivity of these fungi. This assay did not permit an evaluation of the relative importance of each of the potential propagules of VAM fungi to initiate VAM infection. The vertical distribution of living spores within the two soil depths was investigated to gain some insight into the relative importance of spores as a source of inoculum. The large soil-borne spores of VAM fungi are considered by many to be the most important type of VAM inoculum, but their numbers are often poorly correlated with mycorrhizal formation in soil (Brundrett 1991), and their germination potential varies at different times of the year (e.g. Tommerup 1983a; Gemme and Koske 1988). Few viable spores were found in this study. Low densities of living spores have been found in previous surveys of VAM fungi spore populations in a range of ecosystems (e.g. Mosse and Bowen 1968; Read et al. 1976; Abbott and Robson 1977; Janos 1980; Gay et al. 1982; Visser et al. 1984; Brundrett and Kendrick 1988; McGee 1989). Although each spore potentially represents an infective unit, their low density in the soil profile suggests that mycorrhizal infectivity will depend more heavily on infective mycorrhizal roots and possibly on the presence of an intact mycelial network (Jasper et al. 1991).

Disturbances involving the removal and storage of the surface layers of the soil profile have been shown to decrease the infectivity of VAM fungi (Abbott and Robson 1991). In the present study, VAM fungi were demonstrated to be most infective in the upper layer of the intact soil profile. Thus, the potential exists for these fungi to be adversely affected by topsoil disturbance. It seems reasonable to expect that seedlings recolonising subsoil or returned topsoil will be forced to grow in a soil environment with markedly different VAM inoculum levels to the pre-disturbance situation (e.g. Stahl et al. 1988). Field-based experiments examining plant responses to different levels of VAM infection are necessary to clearly demonstrate the functional significance of VAM in the revegetation of disturbed plant communities.

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References

Abbott LK, Robson AD (1977) The distribution and abundance of vesicular-arbuscular endophytes in some Western Australian soils. Austr J Bot 25:515–522

Abbott LK, Robson AD (1991) Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. Agric Ecosyst Environ 35:121–150

Allen SE, Grimshaw HM, Parkinson JA, Quarmby C (1974) Chemical analysis of ecological materials. Blackwell, Oxford

Allen SE, Grimshaw HM, Rowland AP (1986) Chemical analysis. In: Moore PD, Chapman SB (eds) Methods in plant ecology. Blackwell, Oxford, pp 285–344

Ambler JR, Young JL (1977) Techniques for determining root length infected by vesicular-arbuscular mycorrhizae. Soil Sci Soc Am J 41:551–556

Ball DF (1986) Site and soils. In: Moore PD, Chapman SB (eds) Methods in plant ecology. Blackwell, Oxford, pp 215–284

Beadle NCW (1954) Soil phosphate and the delimination of plant communities in Eastern Australia. Ecology 35:370–375

Beadle NCW (1962) Soil phosphate and the delimination of plant communities in Eastern Australia II. Ecology 43:281–288

Bellgard SE (1991) Mycorrhizal associations of plant species in Hawkesbury sandstone vegetation. Austr J Bot 39:357–364

Black R, Tinker PB (1979) The development of endomycorrhizal root systems. II. Effect of agronomic factors and soil conditions on the development of vesicular-arbuscular mycorrhizal infection in barley and on the endophyte spore density. New Phytol 83:401–413

Bradshaw AD (1983) The reconstruction of ecosystems. J Appl Ecol 20:1–17

Brundrett MC (1991) Mycorrhizas in natural ecosystems. Adv Ecol Res 21:171–313

Brundrett MC, Kendrick B (1988) The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. Can J Bot 6:1153–1173

Buchholtz K, Motto H (1981) Abundance and vertical distributions of mycorrhizae in Plains and Barrens forest soil from the New Jersey Pine Barrens. Bull Torrey Bot Club 108:268–271

Cavanagh T (1987) Germination of hard-seeded species (order Fabales). In: Langkamp P (ed) Germination of Australian native plant seed. Inkata Press, Melbourne, pp 58–201

Cochran WG, Cox GM (1957) Experimental designs, 2nd edn. Wiley, New York

Colwell JD (1965) The estimation of phosphorus fertiliser requirements of wheat in southern NSW by soil analysis. Austr J Exp Agric Anim Husb 3:190–197

Daniels BA, Trappe JM (1980) Factors affecting spore germination of the vesicular-arbuscular mycorrhizal fungus, *Glomus epigaeus*. Mycologia 72: 457–471

Fairchild GL, Miller MH (1988) Vesicular-arbuscular mycorrhizas and the soil disturbance-induced reduction of nutrient absorption in maize. II. Development of the effect. New Phytol 110:75–84

Gay PE, Grubb PJ, Hudson HJ (1982) Seasonal changes in the concentrations of nitrogen, phosphorus and potassium, and in the density of mycorrhiza in biennial and matrix-forming perennial species of closed chalkland turf. Ecology 71:571–593

Gemme JN, Koske RE (1988) Seasonal variation in spore abundance and dormancy of *Gigaspora gigantea* and in mycorrhizal inoculum potential of a dune soil. Mycologia 80:211–216

Guildon A, Tinker PB (1983) Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants. I. The effect of heavy metals on the development of vesicular-arbuscular mycorrhizas. New Phytol 95:247–261

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic Press, London

Hazelton PA, Tille PJ (1990) Soil landscapes of the Wollongong-Port Hacking 1:100000 Sheet. Soil Conservation Service of NSW, Sydney, Australia

Hesse PR (1971) A textbook of soil chemical analysis. Murray, London

Janos DP (1980) Mycorrhizae influence tropical succession. Biotropica 12:56–95

Jasper DA, Robson AD, Abbott LK (1987) The effect of surface mining on the infectivity of vesicular-arbuscular mycorrhizal fungi. Austr J Bot 35:641–652

Jasper DA, Robson AD, Abbott LK (1988) Revegatation in an iron-ore mine – nutrient requirements for plant growth and the potential role of vesicular-arbuscular (VA) mycorrhizal fungi. Aust J Soil Res 26:497–507

Jasper DA, Abbott LK, Robson AD (1989a) The loss of VA mycorrhizal infectivity during bauxite mining may limit the growth of *Acacia pulchella* R.Br. Aust J Bot 37:33–42

Jasper DA, Abbott LK, Robson AD (1989b) Soil disturbance reduces the infectivity of the external hyphae of vesicular-arbuscular mycorrhizal fungi. New Phytol 112:93–99

Jasper DA, Abbott LK, Robson AD (1989c) Hyphae of a vesicular-arbuscular mycorrhizal fungus maintain infectivity in dry soil, except when the soil is disturbed. New Phytol 112:101–107

Jasper DA, Abbott LK, Robson AD (1991) The effect of soil disturbance on vesicular-arbuscular mycorrhizal fungi in soils from different vegetation types. New Phytol 118:471–476

Johnson PN (1977) Mycorrhizal Endogonaceae in a New Zealand forest. New Phytol 78:161–170

Koide RT, Mooney HA (1987) Spatial variation in inoculum potential of vesicular-arbuscular mycorrhizal fungi caused by the formation of gopher mounds. New Phytol 107:173–182

Kormanick PP, Bryan WC, Schultz RC (1980) Procedures and equipment for staining large numbers of plant root samples for endomycorrhizal assay. Can J Microbiol 26:536–539

Koslowsky SD, Boerner REJ (1989) Interactive effects of aluminium, phosphorus and mycorrhizae on growth and nutrient uptake of *Panicium virgatum* L. (Poaceae). Environ Pollut 61:107–125

Loree MAJ, Williams SE (1987) Colonisation of western wheatgrass (*Agropyron smithii* Rydb.) by vesicular-arbuscular mycorrhizal fungi during the revegetation of a surface mine. New Phytol 106:734–744

McGee PA (1986) Mycorrhizal associations of plant species in a semiarid community. Aust J Bot 34:585–593

McGee PA (1989) Variation in propagule numbers of vesicular-arbuscular mycorrhizal fungi in a semi-arid soil. Mycol Res 92:28–33

Mertz SM, Heithaus JJ III, Bush RL (1979) Mass production of axenic spores of the endomycorrhizal fungi *Gigaspora margarita*. Trans Br Mycol Soc 72:167–169

Moorman T, Reeves FB (1979) The role of endomycorrhizae in revegetation practices in the semi-arid West. II. A bioassay to determine the effect of land disturbance on endomycorrhizal populations. Am J Bot 66:14–18

Mosse B (1973) Advances in the study of vesicular-arbuscular mycorrhizas. Annu Rev Phytopathol 11:171–196

Mosse B, Bowen GD (1968) The distribution of *Endogone* spores in some Australian and New Zealand soils, and in an experimental field soil at Rothamsted. Trans Br Mycol Soc 51:485–492

Parke JL, Linderman RG, Trappe JM (1983) Effect of root zone temperature on ectomycorrhiza and vesicular-arbuscular mycorrhiza formation in disturbed and undisturbed soils of southwest Oregon. Can J For Res 13:657–665

Porter WM (1979) The 'most probable number' method for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil. Aust J Soil Res 17:515–519

Powell CLL (1976) Development of mycorrhizal infections from *Endogone* spores and infected root segments. Trans Br Mycol Soc 66:439–445

Read DJ, Koucheki HK, Hodgson J (1976) Vesicular-arbuscular mycorrhizae in natural vegetation systems. I. The occurrence of infection. New Phytol 77:641–653

Rives CS, Bajwa MI, Liberta AE, Miller RM (1980) Effects of topsoil storage during surface mining on the viability of vesicular-arbuscular mycorrhiza. Soil Sci 129:125–126

Schenck NC, Graham SO, Green NE (1975) Temperature and light effect on contamination and spore germination of vesicular-arbuscular mycorrhizal fungi. Mycologia 67:1189–1192

Schwab S, Reeves FB (1981) The role of endomycorrhizae in the revegetation practices in the semi-arid West. III. Vertical distribution of vesicular-arbuscular mycorrhiza inoculum potential. Am J Bot 68:1293–1297

Slankis V (1974) Soil factors influencing formation of mycorrhizae. Annu Rev Phytopathol 12:437–457

Smith SE, Bowen GD (1979) Soil temperature, mycorrhizal infection and nodulation of *Medicago trunculata* and *Trifolium subterraneum*. Soil Biol Biochem 11:469–473

Smith TF (1978) A note on the effect of soil tillage on the frequency and vertical distribution of spores of vesicular-arbuscular endophytes. Aust J Soil Res 16:359–361

Stahl PD, Williams SE, Christensen M (1988) Efficacy of native vesicular-mycorrhizal fungi after severe soil disturbance. New Phytol 110:347–354

Tommerup IC (1983a) Spore dormancy in vesicular-arbuscular mycorrhizal fungi. Trans Br Mycol Soc 81:37–45

Tommerup IC (1983b) Temperature relations of spore germination and hyphal growth of vesicular-arbuscular mycorrhizal fungi in soil. Trans Br Mycol Soc 81:381–387

Visser S, Griffiths CL, Parkinson D (1984) Topsoil storage effects on primary production and rates of vesicular-arbuscular mycorrhizal development in *Agropyron trachycaulum*. Plant Soil 82:51–60

White JA, Munn LC, Williams SE (1989) Edaphic and reclamation aspects of vesicular-arbuscular mycorrhizae in Wyoming Red Desert soils. Soil Sci Soc Am J 53:86–90