ROLE OF MYCORRHIZAS IN THE REGENERATION OF ARID ZONE PLANTS

by

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SUMMARY

Mycorrhizas were observed on about ninety percent of the species of plants of a seasonally arid open mallee community at Ferries McDonald Conservation Park in South Australia. Vesicular-arbuscular mycorrhizas (VAM, about 75%) and ectomycorrhizas (about 30%) were the most common associations with orchid, epacrid and other associations also present. The genus Thysanotus had an unusual mycorrhizal association. Fungi that could form either VAM or ectomycorrhizas on other host plants penetrated the root of Thysanotus to ramify longitudinally in air spaces between the cortex and the epidermis. Infection was associated with increased growth in a low nutrient soil.

Of the fungal species identified from the study area, all have been observed in more temperate climates. Only about forty percent of the plants have been observed in more temperate habitats.

There were few spores of VAM fungi and they were not randomly distributed through the soil. The spores of Acaulospora laevis, Entrophospora infrequens and Glomus albidum were found in the upper soil profile with those of Gigaspora calospora lower in the soil. Occurrence of spores was associated with different mechanisms of survival of the wet/dry cycles of summer, when fresh roots were absent. Spores of Gi. calospora germinated rapidly on soil wetting compared to the spores of fungi from the upper profile. Hyphae of all species emerged rapidly from dried pieces of root. However, VAM fungi from the upper profile became dormant as the soil dried; Gi. calospora was killed by one wet/dry cycle. High temperatures of the surface soil over summer appeared to have little effect on the survival of VAM fungi.

In contrast to perennials that could form VAM and ectomycorrhizas but usually had one system predominating, both types of mycorrhizal systems were commonly found on the annual Podotheca angustifolia throughout the growing season. Successful establishment and spread of VAM and ectomycorrhizas on P. angustifolia was affected by soil temperature and water content.

The rate of germination of propagules of VAM, but not ectomycorrhizal fungi, was

enhanced at 30°C compared to 16° or 20°C. Some fungal species that were common at 16° and 20°, were unable to germinate and infect at 30°C. Rate of spread of infection was also temperature sensitive in some fungi. While the presence of ectomycorrhizas did not affect the germination of propagules of VAM fungi, spread of Acaulospora laevis on roots was decreased by the presence of the ectomycorrhizal fungus Peziza whitei.

Those factors found to influence survival of mycorrhizal fungi and initiation of mycorrhizas on plants at the park may also be observed in more temperate climates, which suggests that aridity at Ferries McDonald Conservation Park had little effect on the functioning of mycorrhizas.

Declaration.

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, no material described herein has been previously published or written by another person except where due reference is made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

12/7/87.

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CHAPTER 1

INTRODUCTION

The biological adaptations of plants (and animals) for growth and survival in dry climates are diverse (Barker & Greenslade, 1982). For instance, Beadle (1952) found germination of seed of species of Atriplex is inhibited by the sodium chloride found in the appendages to the seed. Germination only occurs after salt is leached from the appendages, thus ensuring plant growth in a moist seedbed. An adaptation for more efficient use of soil water is by control of the osmotic potential of leaves. In Atriplex confertifolia (Tor. & Frem.) S. Wats. the solute potential in the leaves changes from -5 MPa in spring to -20 MPa in the dry summer of Utah, U.S.A. (Moore et al 1972).

As most plants are mycorrhizal and mycorrhizas are associated with increased uptake of nutrients (Daft & Nicolson, 1965; Harley & Smith, 1983) and alteration of water relations of plants (Safir & Nelson, 1981), it would seem probable that mycorrhizas are particularly important in the growth of plants in dry climates. However, little is known about fungi of arid soils and mycorrhizal fungi, in particular, have been poorly studied (Trappe, 1981). Arid and semiarid climates provide adverse conditions for the survival of mycorrhizal fungi just as they do for plants. Rainfall of irregular intensity and distribution as well as high soil temperatures and cessation of plant growth during summer suggest that only fungi with suitable adaptations would survive. Little is known about` survival of mycorrhizal fungi in dry climates.

Mycorrhizas are symbiotic associations between the roots of plants and some soil borne fungi. There are several different types of mycorrhizas: vesicular-arbuscular mycorrhizas (VAM) and ectomycorrhizas occur on a wide range of host plants. Orchid, epacrid, arbutoid and monotropoid mycorrhizas are confined to single families or genera within a family. Arbutus and Monotropa are not indigenous to Australia and are not considered further. As well, plants in the Orchidaceae and Epacridaceae are rare in arid regions of Australia (Jessop, 1981). Trappe (1981) and Gianinazzi - Pearson &

Diem (1982) suggest that VAM are far more common than other types of mycorrhizas in arid and semiarid regions. However, ectomycorrhizas have been observed in many families that have species that occur in arid regions i.e. Cistaceae, Fagaceae, and Pinaceae (Trappe, 1981), Myrtaceae, Casuarinaceae, Rhamnaceae, Mimosaceae, Fabaceae, Euphorbiaceae, Sterculiaceae, Thymelaeaceae, Apiaceae, Rubiaceae, Goodeniaceae, Stylidiaceae (Warcup, 1980) and Asteraceae (Warcup & McGee, 1983). The majority of plants that form ectomycorrhizas may also form VAM. Families in which arid zone members are usually nonmycorrhizal include Chenopodiaceae, Cyperaceae, Zygophyllaceae and Brassicaceae (Trappe, 1981).

Fungi that form VAM occur in five genera, Acaulospora, Entrophospora, Gigaspora, Glomus and Sclerocystis, of the Endogonaceae (Zygomycetes). The genera differ in the morphology and the development of spores in soil and roots. In all genera, spores may be found singly and in Glomus, Sclerocystis and Acaulospora some species form aggregates of spores in sporocarps. There are few studies of the distribution of VAM fungi in soils of dry regions. Gianinazzi - Pearson & Diem (1982) recorded spores of Gigaspora, Glomus and Sclerocystis, but not Acaulospora in semiarid tropical regions of northern Africa. Mosse & Bowen (1968) reported the presence of only yellow vacuolate and funnel shaped spores from some desert regions of Australia, suggesting that there is a limited VAM fungal flora in arid soils. Of the species of VAM fungi described from arid regions, Glomus deserticola Trappe, Bloss & Menge appears restricted to desert regions. More surveys of VAM fungi need to be completed to determine if other species are confined to arid regions.

Of the fungi that form ectomycorrhizas, members of Terfeziaceae are commonly found in dry habitats, for instance the desert truffles Terfezia and Tirmania from North Africa (Trappe, 1971). Desert truffles have also been recorded from southern Africa, central Australia and south western U.S.A. but little is known about their mycorrhizal associations (Trappe, 1981). Again, few surveys have been made and more information is required before it is known if there is a population of mycorrhizal fungi found preferentially in arid soils.

For mycorrhizas to occur on plants in arid regions, the mycorrhizal fungi must

be able to survive any periods of stress, then germinate when young roots are present and later form survival structures before the cessation of plant growth. Two common stresses of aridity in Australia are periodic lack of water in the soil and high soil temperatures particularly at the surface during summer.

High temperatures of soil may contribute to the death of propagules of fungi. Warcup (1983) examined the effect of sunbaking a forest soil experimentally and found that exposure to full sunlight lowered fungal numbers markedly in the soil. There was also a change in the fungi that formed ectomycorrhizas on seedlings of Eucalyptus regnans after sunbaking, with basidiomycetes and Cenoccum ~~graniforme~~ geophilum being common on plants grown in untreated soil and absent on plants grown in the sunbaked soil where an ascomycete and an Endogone were common. Similar results were found after treating the soil with aerated steam. Here, VAM were also rare in E. regnans grown in soil treated with aerated steam at 71^{o}C for 30 minutes (Warcup, 1981), though not in plants grown in untreated soil. These data suggest that there may be a reduction in the number of propagules of VAM fungi which survive exposure to high temperatures at the soil surface in the field during summer.

This work set out to investigate the importance of mycorrhizas in a dry habitat, the mycorrhizal fungi, especially the VAM fungi, present in an arid soil and the effect of aridity on the survival of mycorrhizal fungi and the initiation of mycorrhizas.

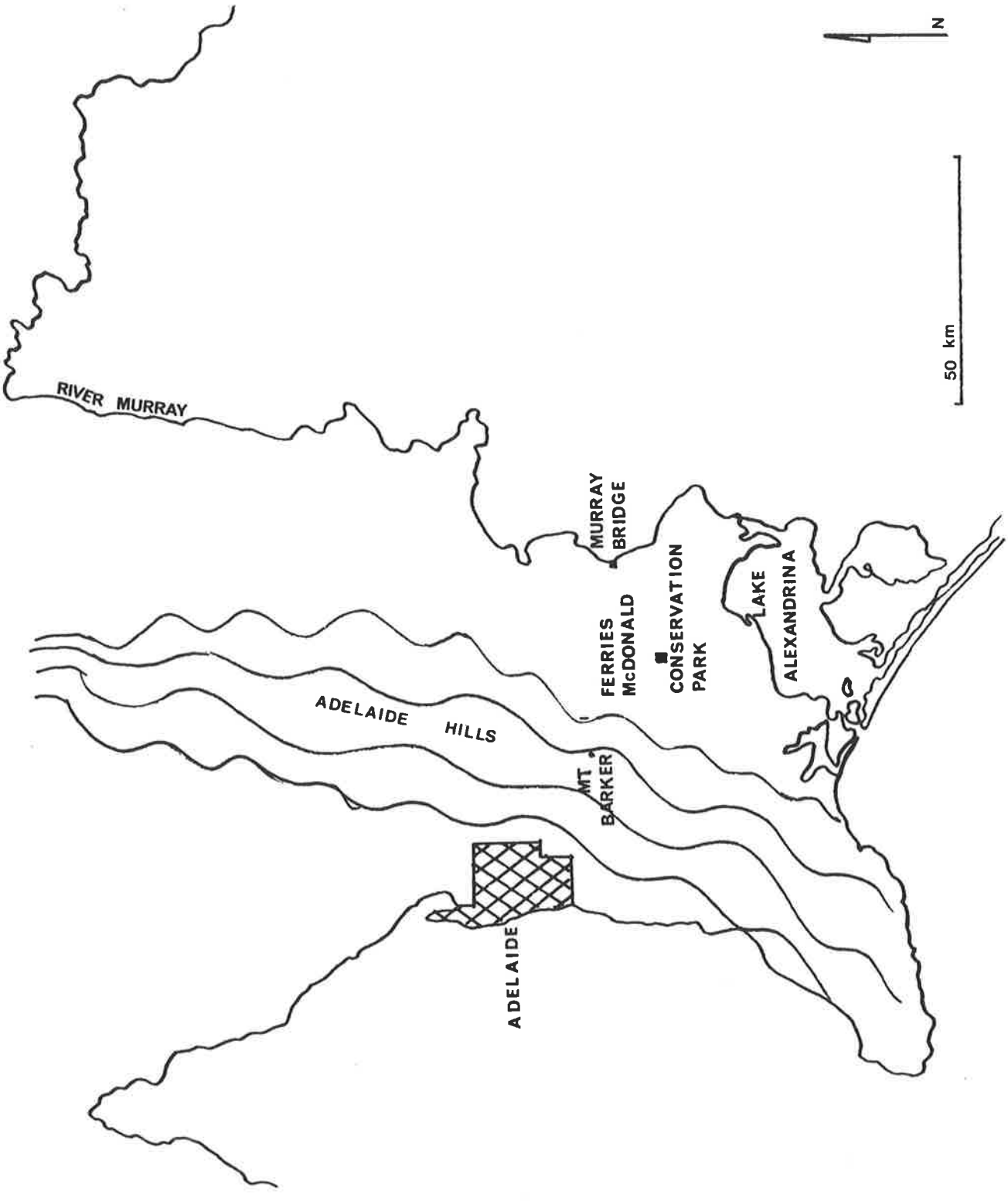
Chapter 2

THE EXPERIMENTAL SITE

The site chosen for investigation and from which soil was obtained for experimental studies was within Ferries McDonald Conservation Park, South Australia. The park is approximately 900 ha and is situated between the Adelaide Hills and the Murray River (Fig. 1) and is in a rain shadow zone caused by the Adelaide Hills lying in the path of the prevailing westerly rain-bearing winds. Farming land is found to the south, east and west of the park with further natural vegetation in the north. The surrounding farms are used mostly for grazing by sheep but in some years cereal crops are sown. A large proportion of the natural vegetation to the north is grazed also by sheep and cattle, as was the park prior to 1960 when the area was privately owned. The park is controlled by the Parks and Wildlife Service of the Department of Environment and Planning of South Australia. While the public is allowed access, the park is distant from urban areas, visitors are infrequent and disturbance caused by humans is minimal.

The vegetation of the park is an open scrub dominated by Eucalyptus incrassata and Melaleuca uncinata (Boomsma & Lewis, 1980) with a diverse understory of shrubs and herbs. The open canopy of the dominant species covers about fifty percent of the ground. Open areas between the dominant species are sparcely vegetated by low growing shrubs and herbaceous annuals. Grasses are rare and Cyperaceae and Juncaceae occur mostly in the low lying areas. More than 250 plant species have been found in the area (Appendix I) though not all annuals are evident every year. The park has an undulating topography and the maximum height of trees is approximately 7m in the hollows and 4m on the more exposed ridges. Trees of Eucalyptus and Melaleuca are mostly found in a mallee form with several trunks arising from a lignotuber at the soil surface. About forty percent of the plant species found at the park occur in the arid regions of central Australia (Specht, 1963) though more than forty percent also occur in the more temperate Adelaide Hills.

Fig. 1 Ferries McDonald Conservation Park, South Australia, in relation to the Adelaide Hills and River Murray.



The park is a block situated longitudinally across two sand dunes. The sand dunes have an approximate east/west orientation (Fig. 2) and overlie limestone. Rainfall drains from the dunes into the depressions and is absorbed there. The sandy soil has a low water holding capacity and the poor soil water characteristics were accentuated by choosing a study site on the upper slope of a free draining sand dune.

The soil of the park is a siliceous fine sand (Uc 1.21, Northcote, 1979) with a neutral to acid surface layer. The sand is at least five metres deep in the depressions. There are low levels of mineral nutrients in the soil. Organic matter has accumulated in the upper layers, particularly under perennial plants. A partial litter layer is found on the soil at the base of trees and shrubs and is composed of leaf and twig debris. The litter layer covers approximately forty percent of the soil surface and is rarely deeper than ten centimetres. Soil under litter is often hydrophobic at the end of summer.

The park has a winter dominant rainfall with summer drought. The summers are hot and winters mild. Rainfall records for 1961 to 1985 (Table 1 & Appendix 2) at the property 'Rocklee' immediately south of the park show an average annual rainfall of 294 mm but range from 153mm in 1972 to 432 mm in 1983. Summer (December to March) is the driest period of the year with fewer days during which rain falls and lower average rainfall each month.

Rainfall and ambient temperature determines the growth period of annual plants. Growth of seedlings commenced after the opening rains, which varied from mid March (1983) to early May (1984). The length of the growing season is also dependant upon rainfall and rising temperatures in spring. In 1983, annuals survived till late October but were dead by early September in 1984 and 1985.

During summer soil temperatures at the surface (Figure 3 & Appendix 3) commonly reach 60 - 70^{o}C in the absence of litter. Under litter temperatures at the soil surface are reduced. Over summer there are also fluctuations of up to 50^{o}C at the soil surface. During the growing season there is considerable variation in soil temperature with both depth of soil and season (Table 2).

Ferries McDonald Conservation Park is a relatively undisturbed area subject to a

Fig. 2. Topographic map of Ferries McDonald Conservation Park showing the study area and the site for the collection of bulk soils.

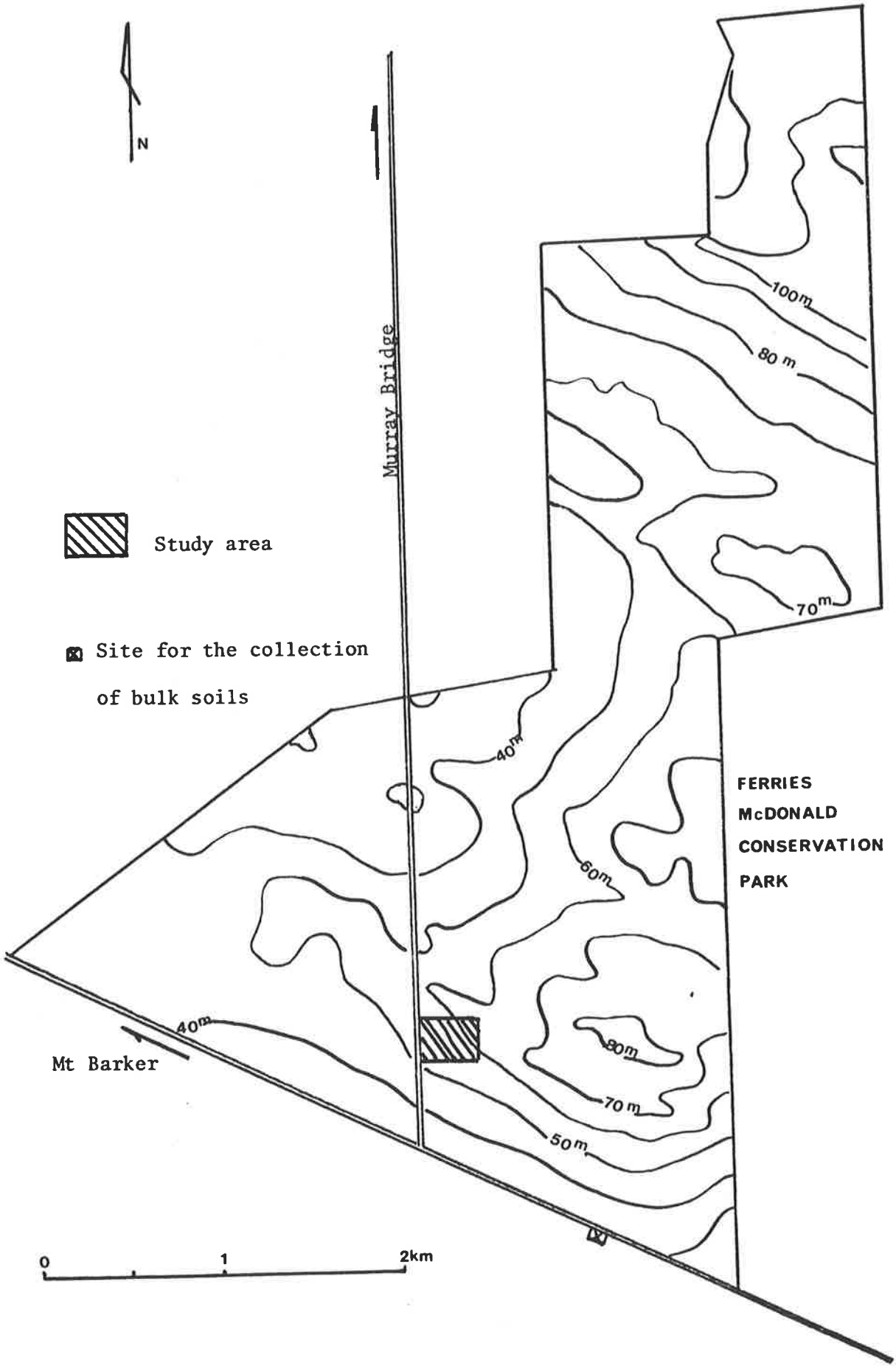


Table 1. Mean monthly number of wet days and rainfall recorded at the southern boundary of Ferries McDonald Conservation Park (1961 - 1985).

Month	Mean number of wet days	Mean rainfall (mm)
January	1.3	16.6
February	1.3	18.0
March	2.4	16.7
April	3.6	29.0
May	5.5	29.0
June	5.1	27.2
July	6.0	29.0
August	6.6	32.8
September	5.2	29.5
October	4.5	29.7
November	2.9	21.6
December	2.3	13.2
Total	46.4	292.0

Fig. 3. Maximum temperature of soil at (a) 2 - 4 and 10 - 12cm deep from 19.1.84 to 29.2.84 and (b) 2 - 4cm deep under a bare surface and under litter from 29.11.84 to 14.2.85.

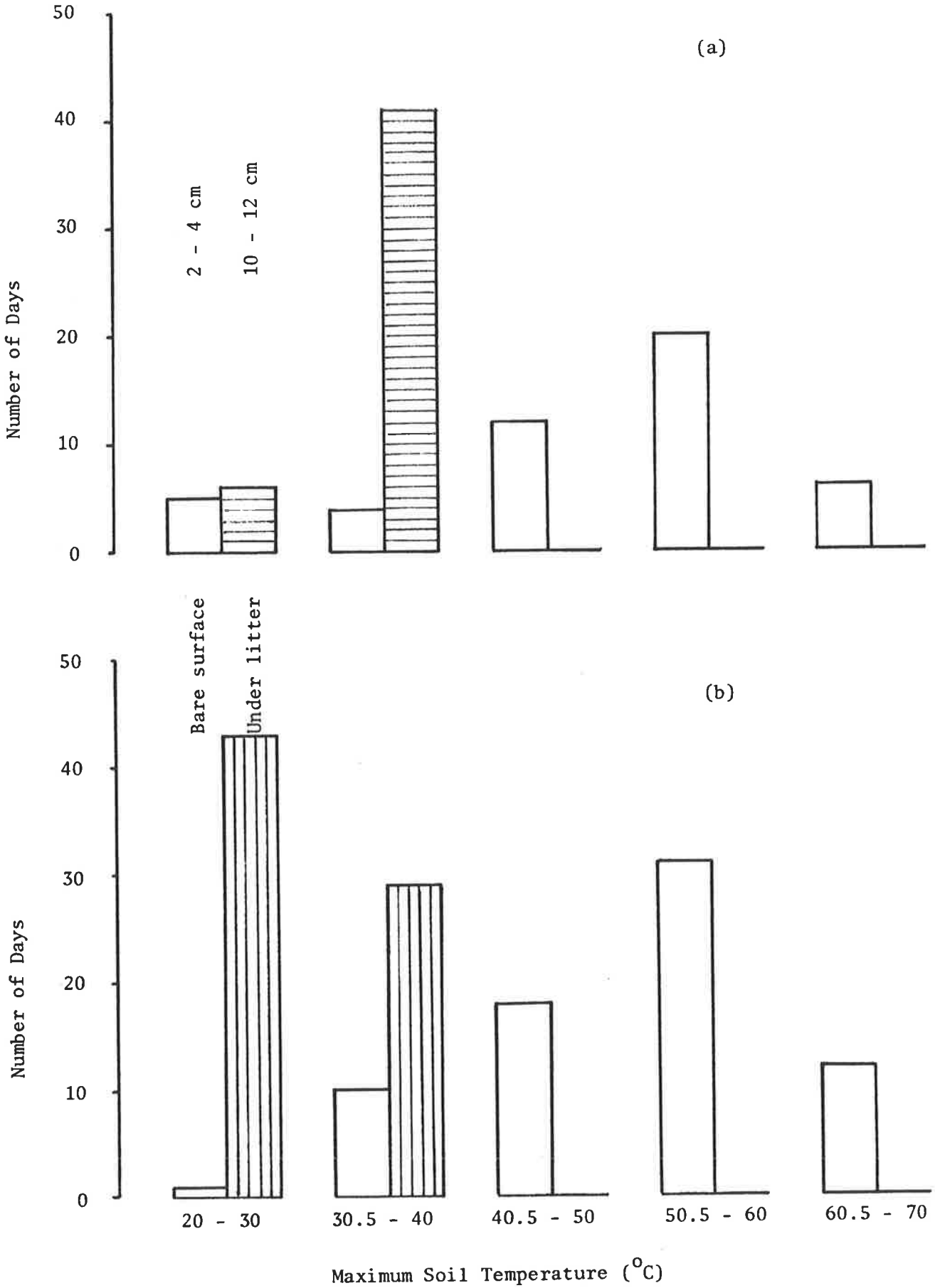


Table 2. Soil temperature (^{o}C) measured during 1983 at six depths either under litter or a bare surface. Mean of three readings.

Depth	Date of observation						
(cm)	10.3	12.5	4.7	27.7	6.9	4.10	6.11
			Bare surface				
0	19*	20	9	10	18	15	27
2	31	23	8	10	18	16	29
5	26	19	7	10	16	16	28
10	20	14	5	8	14	15	24
15	18	12	5	7	13	14	20
20	18	12	6	7	11	13	19
			Under litter				
0	24	19	11	11	16	15	27
2	26	20	8	11	15	15	26
5	25	16	7	10	13	15	22
10	19	13	6	8	12	14	19
15	18	12	7	8	11	13	17
20	18	12	7	7	11	14	17

* - overcast at time of reading.

dry climate with seasonal aridity. Vegetation of the park is diverse with representatives of both arid and temperate habitats present. The soil is free draining, with a low water holding capacity and poor mineral nutrition. The park is also easily accessible from the University of Adelaide. The park is thus a suitable site to examine the role of mycorrhizas in the regeneration of plants in a dry habitat.

CHAPTER 3

MATERIALS AND METHODS

Collection and Preparation of Soils

(chapter 15)

Soil for experiments that required natural inoculum was collected within the study area, 50 to 100m south of the gravel quarry in Ferries McDonald Conservation Park. After removal of surface litter, samples were collected separately either in blocks or loose in bags of approximately 20kg of dry soil. Loose soil was thoroughly mixed with root segments, organic fractions and clods broken and dispersed through the soil. Blocks of soil were collected by pressing a cylindrical pipe, 10cm diameter by 15cm deep, with a sharpened edge, vertically into the soil. Each pipe was then dug out and placed into a plastic lined pot and the pipe lifted out, leaving the soil block in the pot. Pipes closely fitted the pot though when lifted out, some disturbance occurred, particularly at the edge of the block.

for insolation experiments

Bulk soil was collected from south of the park within roadside vegetation (Fig. 2). Bulk soil was usually collected dry from 15 - 30cm deep. On return to the laboratory the collections were sieved through a 2mm sieve and mixed thoroughly, autoclaved and then stored dry in plastic bags for at least six months before use.

Determination of Soil Temperatures in the Field

Continuous recordings of soil temperature were made over the summers of 1983/4 and 1984/5. In 1983/4, a probe were placed at 2 - 4cm and another at 10 - 12cm below the soil surface at a site where litter was absent and the soil was exposed to direct sunlight for most of the day. The recording apparatus was calibrated to the temperature of a mercury thermometer placed in water. Temperatures of the test water were from 21^{o}C to 55^{o}C, the maximum initially anticipated. Field recordings were made from 19.1.84 to 29.2.84. In 1984/5, the probes were placed near the position of the previous year, at 2 - 4cm either under litter or a bare surface. Calibrations were made for temperatures to 70^{o}C.

Recordings were made from 29.11.84 and ceased on 14.2.85 after faults in the recording apparatus made interpretation of the data difficult.

Soil Water

Surface soil was collected from the study site within 24hrs of soaking rain (24.3.83) and the soil water content determined (wet weight minus dry weight divided by the dry weight, multiplied by 100). The soil water content was 10% and this was used as an estimate of the field capacity of the soil. Permanent wilting point was estimated by measuring the water content at -1.5 MPa matric potential. Water content of soil from two profiles within the study site was measured at -1.5 MPa matric potential by Dr T. Dexter, Department of Soil Science, University of Adelaide (Table 3). Estimates of field capacity and permanent wilting point gave an indication of the amount of water available for plant growth. Continued plant growth was expected provided that there was more than about 4.5 - 5% water content in the surface soil and more than about 1.5% in soil from the lower horizon.

Soil water content was calibrated to matric potential using the filter paper method (Hamblin, 1981). Surface soil was moistened to 5, 7.5, 10 or 12% water content with deionised water. Pots of 400g moistened soil were incubated for 24hrs with a filter paper (Whatman No 42) placed in the soil and the matric potential determined from four replicate pots. Water for plant growth was readily available between soil water contents below field capacity to just above permanent wilting point (Table 4), a common occurrence in sandy soils (Greacen and Williams, 1983).

At the start of experiments, soils were moistened to 10% by weight with deionised water (unless otherwise specified). In loose soil, the water was mixed into the soil immediately prior to potting. With blocks of soil, water was added to the surface.

Experimental Plants and Source of Seed

The plants used for mycorrhizal studies and the source of seed of each species (Table 5) was diverse. Initially as seed of suitable species native to the park was

Table 3. Percent water content at -1.5 MPa matric potential of soil samples collected from two profiles within the study site.

	Depth of collection	Water content
Profile 1, under litter	0 - 4cm	4.7%
	12 - 15	1.1
	30 - 40	0.8
Profile 2, bare soil	0 - 4	3.8
	12 - 15	2.1
	30 - 40	1.3

Table 4. Matric potential of soil with 5, 7.5, 10 or 12% water content (Means of four replicates).

% water content	5	7.5	10	12
Water potential (-MPa)	0.008	0.005	0.001	0.001

Table 5. The plant species used in this study.

Plants	Myco. association (1)	Seed size (2)	% germination	Source of seed (3)	Use of plants (4)
Acacia paradoxa*	E,VAM	m	80	S	e p 105
A. longifolia*	E,VAM	m	90	S	e
Betula sp*	E,VAM	m	1	S	e
Blennospora drummondii	VAM	s	80	P	b p. 82
Eucalyptus incrassata	E,VAM	s	70	P	e
E. fasciculosa	E,VAM	s	60	P	e
E. rugosa	E,VAM	s	70	P	e
Helipterum pygmaeum	VAM	s	100	P	b
Melaleuca acuminata	E,VAM	s	70	O	e
M. uncinata	E,VAM	s	70	O	g,c,e
Plantago drummondii	VAM	s	90	G	g
Podotheca angustifolia	E,VAM	s	80	P	g
Quercus sp*	E,VAM	l	70	S	e
Solanum opacum*	VAM	s	90	O	m P. 65
Thysanotus juncifolius	T	m	50	P,O	t P. 116
T. multiflorus*	T	m	50	C	t
T. patersonii	T	m	50	P,O,C	t
T. tenellus*	T	m	60	O	t
Trifolium subterraneum*	VAM	m	90	C	c

* - plants not found at the park. (1) E=ectomycorrhiza, VAM=vesicular-arbuscular mycorrhiza, T=thysanotus, see Chapter 5. (2) l=large, m=medium, s=small. (3) S=collected from Stirling, P=park, O=outside park, G=glasshouse plants, C=commercial sources. (4) b=germination experiments, c=pot cultures, e=initiation of ectomycorrhizas, g= general experiments, m=manganese experiments, t=Thysanotus experiments.

unavailable, Solanum opacum was used as the test plant. Seed germination, infection and growth of ~~this species~~ S. opacum had been previously examined (McGee, 1982). As well, the species is native to soils and climate similar to the park. However, attack by a Rhizoctonia and a Pratylenchus in soil from the park reduced plant growth and the usefulness of S. opacum as a test plant. Similar infections by the Rhizoctonia were also found on Trifolium subterraneum but on this and on plant species native to the park, growth depression did not occur as a result of infection.

Where possible species found at the park were used as mycorrhizal hosts. Where sufficient seed was not available from plants grown on in the glasshouse or from plants from the field, it was obtained from plants outside the park or from commercial sources.

Growth, Inoculation and Treatment of Plants

For most experiments 850g of moistened mixed soil or blocks of 800 to 1400g of moistened soil per pot were used. Seed was sown on the surface of moistened autoclaved sand. Germinated seedlings were transplanted to pots of moistened soil when radicals were 0.5 to 2cm long and when transplanted to mixed soil inoculated with mycorrhizal fungi ~~where appropriate~~. Pots were then placed either in a glasshouse in Wisconsin tanks (waterbaths) set at 16, 20 or 30^{o}C or in a growth room with a 12 hour day, a day temperature of 20 - 22^{o}C and a night temperature of 16 - 18^{o}C. The photosynthetically active radiation in the growth room was approximately 240 µmol m^{-2} sec^{-1} from a bank of 15 Phillips TLF 65/80W33RS white tubes. Pots were watered to weight twice weekly with deionised water.

At harvest, plants were washed from the soil, roots cleared, stained in Trypan Blue (Phillips and Hayman, 1970) and examined microscopically. Root length and length of root infected with VAM fungi were determined by the grid intersect method (Giovanetti & Mosse, 1980). Length of root infected by ectomycorrhizal fungi was determined subsequently. Roots of harvested plants were cut into segments about one cm long. A random sample of 40 root segments from each

plant was placed in glycerol on a slide, aligned parallel to the slide, then covered and examined under a transmitted light microscope. In six random sights across the slide, the number of roots and the number of ectomycorrhizas that passed under the zero of a micrometer scale, were counted. The proportion of roots found to be ectomycorrhizal was determined and the length of ectomycorrhiza estimated. The number of VAM was also counted on these roots, and the proportion of root segments found with VAM checked against the proportion determined by the root intersect method. The results were similar.

Dry weight of shoots was determined after plant material placed in an oven set at 70^{o}C lost no further weight.

Plants were inoculated with mycorrhizal fungi in one of three ways: seedlings became mycorrhizal from natural inoculum in untreated soil; fractions of dry natural soil were placed beneath seedlings transplanted into otherwise autoclaved soil; or seedlings were inoculated with chopped, fresh mycorrhizal roots from a pot culture. Usually, a pad of 0.5g fresh weight of root inoculum was placed one to two cm below the soil surface immediately under the seedling.

Experimental Fungi

The mycorrhizal fungi used in this study and the source of cultures and pot cultures are listed in Table 6. WARH 24 , an ascomycete (Warcup & McGee), was used initially in the Thysanotus experiments, as no suitable ectomycorrhizal ascomycete was available in pot culture. Glomus clarum was used both because it was available in pot culture and because there was a significant response of plant growth to infection in the soil used. Otherwise, fungi that occurred at the park were used.

Establishment of Cultures and Pot Cultures of Mycorrhizal Fungi

Mycorrhizal fungi were isolated from soil or fungal structures and placed in pot culture as soon as practicable, from where they were used experimentally. Isolates of ectomycorrhizal fungi can be made from sporocarps or from ectomycorrhizas. Of the fungi found at the park, FM2 (Amanita sp.) and FM10 (an ascomycete) were isolated from sporocarps on to NDY agar with nutrients at one

Table 6. The mycorrhizal fungi used experimentally in this study.

Fungus	Source
Laccaria ohiensis (Mont.) Singer	Culture collection(1)
Peziza whitei (Gilkey) Trappe	Culture collection(1)
WARH 24*	Culture collection(1)
FM 2	Sporocarp (2)
FM 10	Sporocarp (2)
Acaulospora laevis	Spores (3)
Entrophospora infrequens	Spores (3)
Glomus albidum	Spores (3)
Glomus clarum Nicol. & Schenck*	Soil (4)
Gigaspora calospora	Spores (3)
FE I	Soil (5)

* - fungi not found at the park.

1 = from the culture collection of Dr J.H. Warcup, Dept Plant Pathology University of Adelaide.

2 = Sporocarp collected from the park

3 = Spores collected from soil from the park.

4 = From soil from Loebethal Conservation Park, South Australia.

5 = From soil from the park.

sixth strength and with 1% Streptomycin and 0.1% Tetracycline. Attempts were made to isolate FM Asco I, II and III and the Endogone sp from ectomycorrhizas on seedlings of M. uncinata, P. angustifolia, Podolepis tepperi, Eucalyptus sp and Acacia sp. Only FM Asco III (probably Cenococcum sp) was successfully isolated from P. angustifolia.

Pot cultures of ectomycorrhizal fungi were established by taking a plug of agar from the edge of a growing colony and placing the plug against the root of a seedling of Melaleuca uncinata in a pot of autoclaved soil. Pot cultures of VAM fungi, except for fine endophyte (FE), were established by pipetting spores washed from soil on to the roots of a seedling of M. uncinata in autoclaved soil. The spores of FE I are less than 50μm in diameter, whereas those of most other VAM fungi were larger and do not pass through a 50μm sieve. Pot cultures of FE were obtained by growing a seedling of Plantago drummondii in autoclaved bulk soil inoculated with untreated soil that had passed through a 50μm size sieve. After six weeks growth, the roots from the trap plant were examined for mycorrhizas. After only finding FE in the trap plant, the roots were inoculated under further seedlings of P. drummondii for six weeks. Pot cultures of FE I were maintained on T. subterraneum and were established from root pieces of the second P. drummondii, agan after checking for the absence of other VAM fungi. Pot cultures of VAM fungi that do not form spores in the field were not established.

Mycorrhizal fungi were maintained in pot culture in the growth room. Fresh pot cultures were established using roots from previous cultures after checking microscopically the identity of the mycorrhizal fungus. Pot cultures were two to four months old when used in experiments.

Quantification of Mycorrhizal Fungi Using the Most Probable Number Technique

The source and number of infective units of VAM fungi and the source of infection of ectomycorrhizal fungi was unknown. A method to quantify both VAM and ectomycorrhizal fungi was required that gave comparable data. Thus the Most Probable Number (MPN) method (Alexander, 1965; Porter, 1979) was adapted.

The MPN method measures the number of propagules that infect a trap plant

from aliquots of a serial dilution series. In this case, fungi are said to be present when mycorrhizas are observed in the roots of a trap plant that has been grown for a standard time in the test soil.

Soil samples were collected and each was separately and thoroughly mixed. A subsample of 10g of test soil was diluted by autoclaved bulk soil in a one in ten dilution series. Ten replicates of three dilutions were tested with ten uninoculated controls. The method used varied from that of Porter in that seedlings were sown into open tubes (125mm long by 25mm internal diameter, Fig. 4) placed on a bed of moist autoclaved, washed, coarse river sand. The test soil was placed as a band about 1cm below the surface of autoclaved bulk soil. Plantago drummondii or Podotheca angustifolia were used as trap plants. Plants were watered daily and usually harvested after six weeks. The presence of VAM and ectomycorrhizas on roots were noted microscopically at harvest after clearing and staining the roots and the most probable number of each estimated.

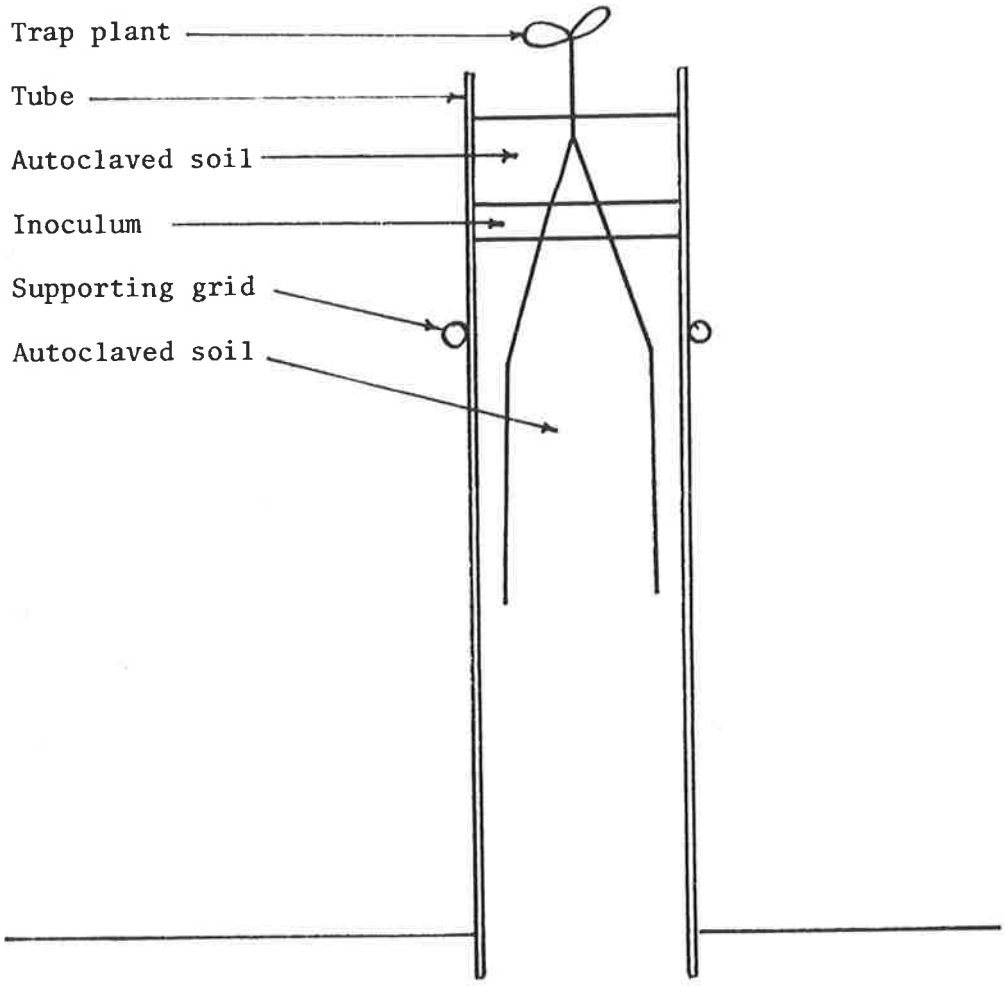


Fig. 4. Diagram of the tube used for plant growth in MPN experiments, showing placement of inoculum. (To scale).

CHAPTER 4

FIELD STUDIES

Mycorrhizal associations typical of dry habitats were expected at the park because of the climate, diverse vegetation and the poor mineral nutrition and free draining nature of the soil. The range of species of plants and mycorrhizal fungi and their associations was examined first. The initiation of mycorrhizas was also investigated, in particular to determine if factors of importance in the regeneration of plants also influenced the germination of mycorrhizal fungi and the formation of mycorrhizas.

Plant Species at the Park

The plant species found in the vicinity of the park were surveyed by Cleland (1955). Not all plants identified by Cleland were found during 1983 - 1986 (Appendix 1). Some of the species may not occur within the park, some may have been misidentified and some may not have had suitable conditions for growth (Parsons & Brown, 1982) during 1983 - 1986. As well, the whole park was not surveyed during the period, and some species may have been missed.

Many plants were identified in the first instance by Mr D. Symon, plant taxonomist, Department of Agronomy, University of Adelaide. Later collections were compared with voucher specimens kept in the laboratory.

Mycorrhizal Fungi at the Park

Collection and Isolation of Mycorrhizal Fungi

During 1983 to 1985 fungal sporocarps were collected while collecting soil and plant material. Sporocarps were returned to the laboratory, identified where possible and isolations attempted. However, many sporocarps at the park dried rapidly in situ and isolations were difficult unless the sporocarps were recently emerged. No further collections were made once a fungus was in culture. Hypogeous sporocarps were difficult to find in the sandy soil; only a limited search was made for them and they may be underrepresented; no Tuberales were collected. Most collections were made during the winter of 1983. Fewer species and numbers of sporocarps were observed in 1984 and 1985. Isolates were not obtained from seven fungi, mostly agarics.

All isolates were tested for their ability to form ectomycorrhizas with M. uncinata. Of the ten fungi in culture only five formed ectomycorrhizas in pot culture (Table 7).

Spores of VAM fungi were wet sieved and decanted from soil using 750, 250 and 50µm mesh sieves. Spores were identified (Trappe, 1982) where possible (Table 8) and pot cultures established. Of the species obtained, A. laevis, Gi. calospora and G. albidum formed spores in pot culture. E. infrequens was established in pot culture but did not form spores. Gl. fasciculatum and Gl. mosseae were not inoculated into pot culture, the former because it was of limited occurrance and only found outside the study site and the latter because it was found only in alkaline soil at the edge of the park.

A further fungus, FMp, was found. Spores of FMp were globose, 80 - 120µm diameter, with two walls. The outer wall was 6 - 8µm thick, yellow to brown and laminated. The inner wall was 2 - 4µm thick, hyaline and laminated. The fungus resembled Acaulospora and Entrophospora in that the spores were globose and lacked subtending hyphae. However, FMp was usually found in aggregates of two to six spores, without any evidence of mother cells, hyphae or membranous sporangial walls. FMp did

Table 7. Mycorrhizal status of some Higher fungi isolated from sporocarps at Ferries McDonald Conservation Park.

Collection	Fungus	Mycorrhizal Status
FM 1	Polyporus basilapiloides	-
2	Amanita sp	+
4	Thelephora sp	-
5	Peziza austrogeaster	-
7	Amanita sp	-
8	Laccaria ohiensis	+
9	Peziza whitei	+
10	Ascomycete (Pezizales)	+
15	fawn agaric	+
17	Gasteromycete	-

+ : forms ectomycorrhizas

- : no mycorrhizas observed in tests.

Table 8. Vesicular-arbuscular mycorrhizal fungi recovered as spores from Ferries McDonald Conservation Park with maximum spore numbers per 100g dry soil.

Species	Maximum number of spores per 100g soil.
Acaulospora laevis Gerd. & Trappe	25
Entrophospora infrequens Ames & Schneider	58
Gigaspora calospora (Nicol. & Gerd.) Gerd. & Trappe	42
Glomus albidum Walker & Rhodes	35
Glomus fasciculatum (Thaxter sensu Gerd.) Gerd. & Trappe	5
Glomus mosseae (Nicol. & Gerd.) Gerd. & Trappe	2
FMp	5

not form mycorrhizas in pot culture and thus its status as a mycorrhizal fungus is unclear.

Vesicular-arbuscular endophytes with fine hyphae were noted during examination of stained roots. Two different fungi were present, FE I and FE II. FE II was not obtained in pot culture. FE I formed spores in association with M. uncinata in pot culture. The spores were globose, 25 - 35µm diam., hyaline, with two walls (Fig. 5). The outer laminated spore wall was approximately 1 - 1.5µm thick. The inner wall was membranous and sealed the contents of the spore. During spore formation, the subtending hypha was swollen to about 10µm diam. and had thin walls (Fig. 6). The subtending hypha subsequently collapsed and often detached from the spore near the spore wall. The fungus appears to be an undescribed species.

A number of other VAM fungi were also observed as infections in roots but no spores were obtained from soils from the field or pot cultures.

Number of Propagules of VAM Fungi in Soil

VAM fungi can survive in soil as spores or as hyphae in roots. The quantity of spores and the total number of propagules and the importance of each in the initiation of VAM was determined.

Spores.

Initially, samples of soil were collected from throughout the park in 1983 and then the study site from 1984 to 1986, usually when the soil was dry. Samples from the study site were collected from under litter and from bare areas. Spores of VAM fungi were wet sieved from 100g of each sample of soil and examined microscopically in a nematode counting dish.

The species observed and the range of numbers of spores of each species for three collections at two depths during the summers of 1983/4, 1984/5 and 1985/6 are given in Table 9. There were similar trends observed each year. There were more spores found in the surface soil than at depth. There was stratification of species according to the spores found, with medium sized spores in the surface soils and the

Fig. 5. Spore of FE I showing the two walls.Subtending hypha is obscured by the spore. 1cm = 14um

Fig. 6. Immature spore of FE I showing the inflated subtending hypha . 1cm = 14um

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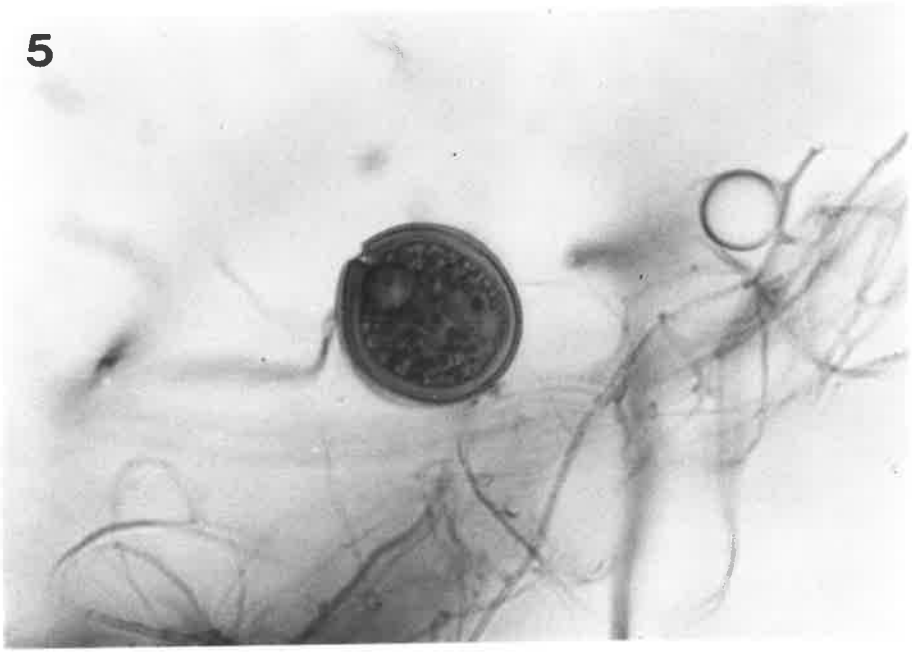

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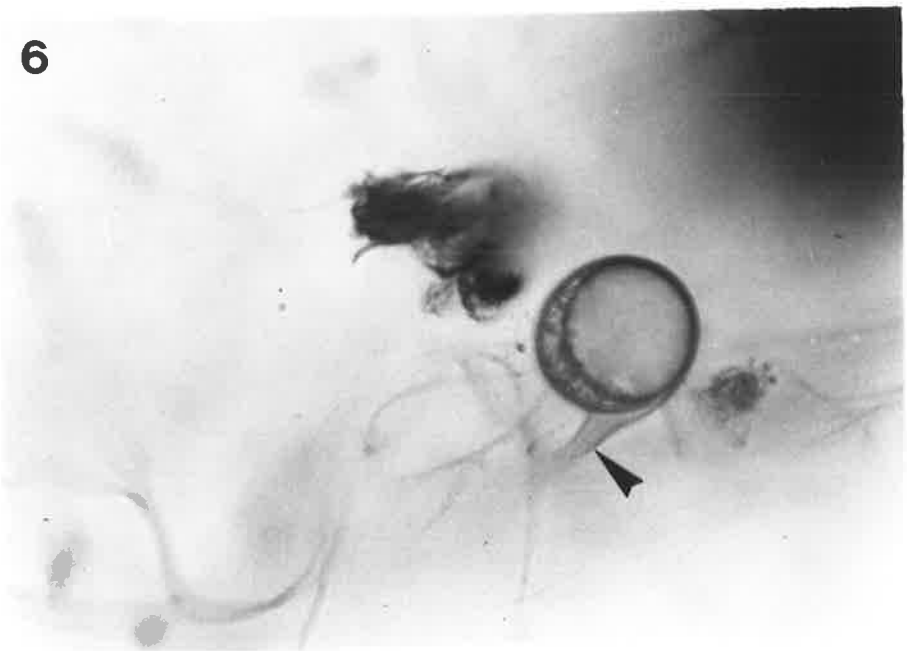


large spored Gigaspora calospora usually the predominant species found in deeper samples.

Random soil samples were also collected from within the park outside the study area. The species collected and the highest counts of spores per 100g dry soil are given in Table 8. Total counts of spores are low compared with many agricultural soils which may range from 14 - 1952 per 100g dry soil (Hayman & Stovold, 1979).

Other propagules.

Although widely used, counts of the number of spores per 100g dry soil do not give an accurate indication of the number of propagules of VAM fungi that occur in a soil. This is because spores are not the only propagules found in soil (Baylis, 1969; Tommerup & Abbott, 1981), not all spores are recovered from soil (Hall, 1977), and spore counts do not give an accurate indication of the viability of spores.

For these reasons a Most Probable Number (MPN) method to estimate the number of infective units in soil has been developed (Alexander, 1965; Porter, 1978). Values obtained by this method are dependant on the conditions of the experiment. For example, the rate of growth of fungal hyphae and roots varies with temperature thus affecting their interception. By providing uniform conditions for each experiment, however, comparable results can be obtained (Wilson & Trinick, 1983A). Soil samples were collected from the surface (0 - 1cm), from the zone above where soil colour changed from dark to light (about 10cm) and from deeper in the soil (30 - 40cm). MPN estimates of propagule numbers of the soil samples were obtained (Table 10; see also Tables 24, p93, 25, p 96 and 27, p106). Maximum density of propagules occurred near the soil surface and decreased down the soil profile.

Table 9. Numbers of spores in 100g dry soil collected during summers of 1983/4, 1984/5 and 1985/6 from the soil surface or 20 - 30cm deep, either from under litter or from bare areas.(Range of three samples).

Depth cm	Year	Fungal species					
		A. laevis	E. infrequens	G. albidum	Unidentified	Gi. calospora	Total
				Under Litter			
0-10	83/4	0 - 18	3 - 30	0	0 - 2	0	4 - 35
	84/5	0 - 5	2 - 10	0 - 3	0 - 5	0	5 - 22
	85/6	0	1 - 8	0 - 1	0 - 1	0	2 - 10
Mean no spores		3	9	1	1	0	14
20-30	83/4	0	0	0	0	0 - 1	0 - 1
	84/5	0	0	0	0	1 - 2	1 - 2
	85/6	0	0	0	0	1 - 2	1 - 2
Mean		0	0	0	0	1	1
				Bare Areas			
0-10	83/4	0 - 1	2 - 6	0	0 - 6	0	2 - 8
	84/5	1 - 4	2 - 5	2 - 10	0 - 4	0	4 - 17
	85/6	0 - 5	5 - 13	0	0 - 9	0	7 - 32
Mean		2	5	2	2	0	11
20-30	83/4	0	0	0	0	0 - 1	0 - 1
	84/5	0	0	0	0	0 - 2	0 - 2
	85/6	0	0	0	0	0 - 1	0 - 1
Mean		0	0	0	0	1	1

hyphal diameter mostly less than 1μm

Table 10. Most Probable Number of propagules of FE & CE and Total VAM fungi (calculated separately) per 100g dry soil, with 95% confidence limits, using Plantago drummondii grown for six weeks in soil inoculum collected from 0 - 1cm, 0 - 10cm or 30 - 40cm down a soil profile, March, 1984.

Sample depth	FE	CE	Total
0 - 1 cm	3 (1.2 - 4.8)	0.4 (0.2 - 1.0)	3.8 (1.6 - 8.8)
0 - 10cm	15.6 (6.8 - 36)	4.4 (1.9 - 10.2)	20 (8.6 - 46.4)
30 - 40cm	0.4 (0.2 - 1.0)	3.2 (1.0 - 5.2)	3.8 (1.6 - 8.8)

Number of Propagules of Ectomycorrhizal Fungi

Only one attempt was made to quantify the number of propagules of ectomycorrhizal fungi. In an experiment to examine competition between VAM and ectomycorrhizal fungi on Podotheca angustifolia, there were similar MPN of VAM and ectomycorrhizal fungi (17 and 20 propagules per 100g dry soil, respectively, after four weeks; Table 27, p 106) .

Three problems arose in checking roots for ectomycorrhizas. Should the fungus not stain readily (e.g. FM Asco I) then it was possible to miss an ectomycorrhiza because it was obscured by a densely stained VAM, by the root, when the patch of ectomycorrhiza was entirely on the lower surface of the root or by observational error. These difficulties in observing ectomycorrhizas required examination of roots at a higher magnification than for VAM. Thus the estimation of the number of propagules of ectomycorrhizal fungi in soil may be low.

Mycorrhizal Associations at the Park

Vesicular-arbuscular mycorrhizas are more common than other types of mycorrhizas in many plant communities (Harley & Smith, 1983). One suggestion for the predominance of VAM is that there is an inverse relationship between mycorrhizal type and richness of plant species, with ectomycorrhizas (sensu Harley and Smith) occurring where there is a low diversity of plant species (Malloch, Pirozinski & Raven, 1980). To extend the information on mycorrhizal associations in the field, plants from the park were examined for mycorrhizas.

Roots of plants were collected from autumn, 1983 to spring, 1985. It was intended to gather representatives of as many plant families as possible. Hence some common species were not collected (e.g. Eucalyptus rugosa and E. oleosa) and some comparatively rare species examined.

Roots of perennial and annual plants were examined. At least 20cm of fine feeder root from each perennial plant was collected, usually at flowering, by following a root system from the base of the plant to its outer limits. Roots from at least three plants of each perennial species were examined. Seedlings of annual plants were collected when found throughout the growing season. Lengths of root were removed from the seedlings in the laboratory and where the identity of the host was unknown, the seedling grown on to flowering. Roots of at least ten seedlings of each annual species were examined. All orchids were collected prior to flowering and treated as annuals. They could not be identified to species as plants failed to flower in the glasshouse. Orchids were identified to genus from vegetative parts.

Roots were cleared, stained and examined for mycorrhizas. When necessary, roots were stored in 50% ethanol prior to clearing (Gardner, 1975).

Classification of Mycorrhizas

In this work various morphological types of mycorrhizal associations were observed. The system of classification used had to be valid for young mycorrhizas as well as mature ones. Each association was classified according to the following definitions:

Vesicular-arbuscular mycorrhizas.

Characterised by the presence of arbuscules in the cortical cells of the root. Arbuscules are connected to each other and the soil by aseptate hyphae. Vesicles may be present.

Vesicular associations.

Characterised by the presence in the root of intercellular vesicles that are attached by aseptate hyphae to the soil. No arbuscules are present.

Coiling VAM.

VAM that also have tightly formed coils of hyphae in the epidermal and / or cortical cells of the root. Arbuscules and vesicles may appear malformed.

Ectomycorrhizas.

Presence of a partial or entire sheath or mantle of hyphae with or without a Hartig net on the roots of plants (Warcup, 1980). The fungi may be aseptate or septate.

Epacrid mycorrhizas.

Characterised by the development of extensive coils in the cortical cells of fine roots of members of the Epacridaceae by fine septate hyphae that have connections to the soil (Harley, 1959).

Orchid mycorrhizas.

Characterised by the development of coils in the epidermal or cortical cells of 'roots' of members of the Orchidaceae by fungi with septate hyphae. Coils collapse before the death of the cells (Harley, 1959).

Thysanotus mycorrhizas.

Characterised by the presence of a partial layer of hyphae between the cortex and epidermis of the root of Thysanotus. The fungi may be aseptate or septate.

Non-mycorrhizal plants.

None of the above associations observed.

Table 11. Mycorrhizal associations of plant species from Ferries McDonald Conservation Park.

Cuppressaceae			
C. columellaris	VAM		
Anthericaceae			
Thysanotus jucifolius	Thysanotus	T. patersonii	Thysanotus
Tricoryne elatior	VAM		
Asphodelaceae			
Bulbine semibarbarta	VAM	Laxmannia sessiliflora	VAM
Colchicaceae			
Wurmbea dioica	Coil VAM		
Dianellaceae			
Dianella revoluta	VAM		
Poaceae			
Aira caryophyllea	VAM	Avellinia michelii	VAM + Nil
Danthonia caespitosa	VAM		
Xanthorrhaceae			
L. juncea	Nil		
Aizoaceae			
Carprobrotus modestus	Nil		
Apiaceae			
Daucus glochidiatus	VAM	Hydrocotyle pilifera	VAM
Trachymene(?) pusillus	VAM		
Asteraceae			
Actinobole uliginosum	VAM	Arctotheca calendula	VAM
Blenospora drummondii	VAM	Brachycome ciliarus	VAM
B. lineariloba	VAM	B. perpusilla	VAM
Gnaphalium involucratum	VAM	Helichrysum apiculatum	VAM + Ect
H. leucopsidium	VAM + Ect	H. obtusifolium	VAM
Helipterum pygmaeum	VAM	Millotia tenuifolia	VAM
Olearia ciliata	VAM	O. ramulosa	VAM
Podolepis rugata	VAM + Ect	P. tepperi	VAM + Ect
Podotheca angustifolia	VAM + Ect		
Pseudognaphalium luteo-album	VAM		
Senecio glossanthus	VAM	S. lautus	VAM
Toxanthus muelleri	VAM + Ect	Vittadinia dissecta	VAM
Brassicaceae			
Brassica tournefortii	V + Nil	Cardamine	Nil
Stenopetalum lineare	V + Nil		
Campanulaceae			
Wahlenbergia communis	VAM		
W. gracilenta	VAM		
Caryophyllaceae			
Petrorhagia velutina	Nil	Silene nocturna	V
Casuarinaceae			
Allocasuarina muelleriana	VAM + Ect		
Crassulaceae			
Crassula colurata	Nil		
Dillenaceae			
Hibbertia stricta	VAM		
Droseraceae			
Drosera macrantha	Nil		
Epacridaceae			
A. humifusum	VAM + Ect		
Leucopogon clelandii	Epacrid		
Euphorbiaceae			
Beyeria leschenaultii	VAM		
Poranthera microphylla	VAM + Ect		
Fabaceae			
Dillwinia hispida	VAM + Ecto		

Gentianaceae			
Centaurium erythrea	Coil VAM		
Goodeniaceae			
Dampiera rosemarinifolia	VAM + Ect	Goodenia affinis	VAM + Ect
G. robusta	VAM + Ect		
Haloragaceae			
Glischrocaryou behrii	VAM		
Lamiaceae			
Prostanthera aspalathoides	VAM		
Westringia dampieri	VAM		
Mimosaceae			
A. rigens	VAM + Ect		
A. spinescens	VAM		
Myrtaceae			
Baeckea behrii	VAM	B. crassifolia	VAM + Ect
Calytrix tetragona	VAM + Ect	Eucalyptus fasciculosa	Ect
E. incrassata	VAM + Ect	Leptospermum myrsinoides	VAM + Ect
Melaleuca uncinata	VAM + Ect		
Orchidaceae			
Caladenia sp.	Orchid	Corybas sp.	Orchid
Diurus sp.	Orchid	Pterostylis sp.	Orchid
Thelymitra sp.	Orchid		
Plantaginaceae			
Plantago drummondii	VAM		
Polygalaceae			
Comesperma calymega	VAM + Ect		
Portulucaceae			
Calandrinia granulifera	Nil	C. volubilis	V + Nil
Primulaceae			
Anagallis arvensis	VAM		
Proteaceae			
Grevillea ilicifolia	V + Nil	G. lavandulaceae	Nil
Rhamnaceae			
Cryptandra tomentosa	VAM	Pomaderris obcordata	VAM + Ect
Spyridium eriocephalum	VAM		
Rutaceae			
Boronia coerulescens	VAM	Correa schlechtendalii	VAM
Eriostemon pungens	VAM	Phebalium bullatum	VAM
Scrophulariaceae			
Zaluzianskya divaricata	VAM		
Sterculiaceae			
Lasiopetalum behrii	VAM + Ect		
Thymelaeaceae			
Pimelea glauca	VAM		
Violaceae			
Hybanthus floribundus	VAM		

Mycorrhizal Associations of Plant Species at the Park

Mycorrhizal associations were observed on 93 plant species in 37 families (Table 11) of which 54 species were perennials and 39 were annuals. There were 69 species found with VAM, 23 with ectomycorrhizas (22 with both), two with coiling VAM, five with orchid mycorrhizas, one with epacrid mycorrhizas, two with Thysanotus mycorrhizas, five were found with vesicular associations and no mycorrhizas were found on eight of the plant species examined.

Ectomycorrhizas were found on members of two plant families not previously recorded as having ectomycorrhizal members (Epacridaceae, Astroloma; Polygalaceae, Comesperma). Further, Podotheca and Toxanthes (Inulae: Asteraceae), Dampiera (Goodeniaceae), Baeckea and Calytrix (Myrtaceae) have not been previously recorded as ectomycorrhizal though they are in families in which other genera are known to form ectomycorrhizas. A few species were recorded with only VAM where the species or others in the genus have formed ectomycorrhizas in pot culture (Cryptandra tomentosa, Spiridium parvifolium, S. vexilliferum and Pimelea spathulata; Warcup, 1980) which suggests that field observations at one site may not always give a complete indication of the mycorrhizal associations of some plants.

The mycorrhizal associations of the monocotyledonous genus Thysanotus (Anthericaceae; Dahlgren, Clifford & Yeo, 1985), differed from both ectomycorrhizas and VAM though the fungi of the association could form either on other host plants. The mycorrhizas of Thysanotus were placed in their own group as they did not fit readily into any other group. The association was examined in more detail (Chapter 5).

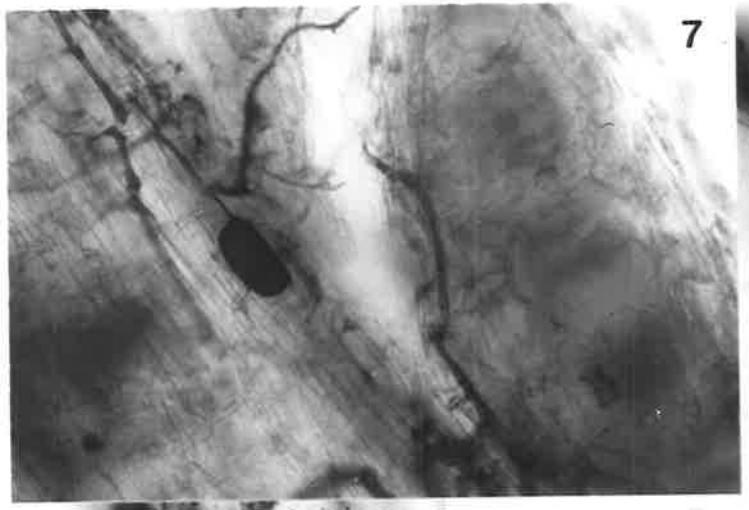
Plants in which vesicular associations were observed all belong to groups previously believed to be free of mycorrhizas. Not all collections of roots from each species contained vesicles. In all cases at the park, vesicular associations were observed in roots in which the epidermis was intact (in the cluster roots of Grevillea ilicifolia, fig. 7; in Brassica tournefortii, Fig. 8). This is contrary to Hirrel, Mehraveran and Gerdemann (1978) who observed vesicular infection in senescing roots of four species

Fig. 7. Vesicle in the cluster root of Grevillea ilicifolia. 1cm = 65um

Fig. 8. Vesicle in a young root of Brassica tournefortii. 1cm = 65um

Fig. 9. Coiling VAM in the root of Centaurium erythraea. 1cm = 28um

Fig. 10. Coiling VAM in the root of Wurmbea dioica. 1cm = 160um



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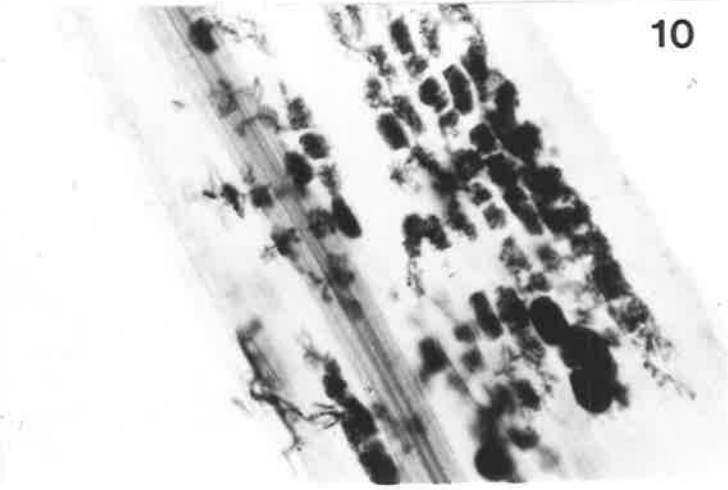

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of Chenopodiaceae and two species of Brassicaceae that had been growing with companion plants with VAM. No evidence was found to suggest that growth of the host was enhanced by the association. In addition, vesicular associations are not a stage of development of VAM subsequent to the collapse of arbuscules. The exact nature of vesicular associations remains unclear.

The grouping of VAM according to the presence or absence of coils may be of little value until the cause of coil formation is more clearly understood. The presence of coils of other taxa of fungi in orchids and epacrids suggests there may be a common phenomenon involved. The formation of coils of hyphae and collapse of hyphal structures in the roots of Centaurium erythraea (Fig. 9) were associated with inhibition of VAM fungi (McGee,1985).

However, in Wurmbea dioica the morphology of the mycorrhizas (Fig. 10) differed significantly from that of C. erythraea with coils formed from branched hyphae in cortical cells, with an absence of immediate collapse of hyphae. The survival of fungi within mycorrhizal roots of W. dioica was examined using roots harvested from plants collected from Stirling, South Australia. Surface hyphae were removed from the roots, the roots chopped and used as inoculum under seedlings of Trifolium subterraneum (McGee, 1985). At harvest, all four replicates of T. subterraneum were found to have VAM and none had coils of hyphae present. Morphology of ectomycorrhizas has been demonstrated to be at least partially under the control of the host (Kope, 1984). Clearly, coiling is, in the case of both W. dioica and C. erythraea, also controlled by the host.

The morphology and physiology of the association differs in W. dioica and C. erythraea. However, the presence of coils may indicate variation in the physiology of the association from commonly studied VAM. Further studies of the mycorrhizas of W. dioica were not possible as seed of the species could not be germinated.

Mycorrhizas are very common at the park. This suggests that mycorrhizas are important for the survival of plants in semiarid environments as in more temperate climates. Alternative forms of nutrient aquisition were observed on some of the non-mycorrhizal plants. For example, parasitic roots were observed on Santalum murrayanum and proteoid roots were observed on Grevillea lavandulaceae. The presence of mycorrhizas or visible alternative forms of nutrient aquisition do not appear to be essential for survival in semi-arid habitats as there were some species without such alternatives, such as Crassula colourata and Lomandra juncea.

Predominance of VAM and Ectomycorrhizas

During the collection of roots to be examined for mycorrhizas, it became apparent that even though some species could form both VAM and ectomycorrhizas, in many cases only one type of mycorrhiza was common on a species. The possibility of predominant associations was examined. Three quadrats, 5m by 5m were randomly chosen at the southern end of the study area. Roots were sampled from all plants in each square during winter, 1984. The results are presented in Table 12.

Some perennials were found with only one type of association. Acacia rigens (four plants), Eucalyptus fasciculosa (two plants) and Melaleuca uncinata (20 plants) were only found with ectomycorrhizas and the perennial herb, Helichrysum leucopsidium (14 plants) only with VAM. Annual species found within the quadrats, capable of forming both VAM and ectomycorrhizas, had both types present, except for one plant of Toxanthes muelleri which had ectomycorrhizas only. Not all roots of this plant were collected and ~~ectomycorrhizas~~ VAM may have occurred on the roots remaining in the ground.

Table 12. Mycorrhizas on plants that can form VAM and ectomycorrhizas found in three quadrats at Ferries McDonald Conservation Park.

Growth habit	Plant Species	Number of plant with Ecto	VAM	Both
Perennial*	Acacia rigens	4	0	0
	Baekea crassifolia	0	1	6
	Eucalyptus fasciculosa	2	0	0
	Helichrysum leucopsidium	0	14	0
	Leptospermum mysinoides	0	0	2
	Melaleuca uncinata	20	0	0
Annual	Podotheca angustifolia	0	0	39
	Toxanthes muelleri	1*	0	118

* - incomplete root system collected.

Temporal Distribution of Mycorrhizas on Podotheca angustifolia

The possibility that the type of mycorrhiza may change with age of a host that can form both VAM and ectomycorrhizas, was examined. Podotheca angustifolia was chosen as a test plant as it occurs widely in the park, was usually found with both VAM and ectomycorrhizas, was found as small or large plants, and was a species which could be readily identified in the field from about five days after the emergence of cotyledons.

At intervals of about two months from early May, 1984, to the end of the growing season in September, a plant of P. angustifolia was harvested from each of five sites in the Park. They were a disturbed area, a south facing slope, a north facing slope, a ridge where rocks were on the surface and a low lying area where sedges, non-mycorrhizal plants, predominated. Roots of harvested plants were examined for mycorrhizas. All plants harvested were found to have both VAM and ectomycorrhizas. At the first harvest, the ectomycorrhizal fungi appeared to be ascomycetes (FM Asco I and II) and later collections had FM Asco III (Cenococcum) as well. No plants with ectomycorrhizas formed by basidiomycetes were found during this study.

Read, Kianmehr and Malibari (1977) observed a succession of fungi, from VAM to ectomycorrhizas on the woody shrub, Helianthemum chamaecistus Mill, and Lapeyrie and Chilvers (1985) on the tree Eucalyptus dumosa Cunn. ex Schauer. While such a succession may occur on some shrubs and trees, there is little evidence to suggest such a succession occurs on annuals at Ferries McDonald Conservation Park.

Discussion

The widespread distribution and predominance of VAM at the park is similar to findings in other ecosystems (Read, Koucheki & Hodgson, 1976; Reeves, Wagner, Moorman & Kiel, 1979; Malloch & Malloch, 1981; Hoberg, 1982; Bethlenfalvay, Dakessian & Pacovsky, 1984). However, the high incidence of ectomycorrhizal associations resembles northern cool habitats (28%, Malloch & Malloch, 1981) and wet tropical habitats (15%, Hoberg, 1982) more than other arid (0%, Bethlenfalvay et al, 1984) habitats that have been surveyed although these authors considered ectomycorrhizas sensu stricto.

From these surveys, it is not clear what factors are of importance in determining whether significant ectomycorrhizal associations occur. Experimental work and further surveys of specific habitats are required to clarify whether the presence of ectomycorrhizas is due to the dry environment or whether some other factor of the southern Australian habitat is of importance. It is clear, however, that ectomycorrhizas may occur where there is a high level of diversity of plant species. Ectomycorrhizas are also associated with a range of plant types, from herbaceous to woody, annual to perennial. At the park, VAM and ectomycorrhizas are found together on one host and even on the same segment of root. Given that some hosts, capable of forming both VAM and ectomycorrhizas, are commonly found with one type, it is likely that the factors governing infection will not be simple and more work is required to determine the conditions necessary for infection by each type of mycorrhizal fungus in the presence of mixed fungal inoculum.

Growth of Roots and Initiation of Mycorrhizas

The initiation of mycorrhizas at the start of the wet season requires the emergence of fresh roots from either seeds or old roots, the germination of fungi from propagules and the subsequent formation of mycorrhizas.

During observation of the initiation of mycorrhizas, there was a need to identify fungal taxa found on or in the roots as different fungi may interact differently with the host. The following criteria were used.

Identification of Fungi in Mycorrhizas

Vesicular-arbuscular mycorrhizas.

The identity of several of the VAM fungi could be determined from the morphology of infections. After checking the morphology of infection of fungi in pot culture, it was possible to clearly separate four taxa: Acaulospora laevis, Entrophospora infrequens, Gigaspora calospora and FE. A fifth taxon, Glomus albidum, was not clearly separate from an unknown group and was therefore excluded in most cases. The fungi were separated on the characters given in the following key, adapted from Abbott and Robson (1978).

I Hyphae mostly < 1µm diameter, dark staining .....FE

Hyphae > 1 µm diameter,variable staining (CE) II

II Hyphae often looped in epidermal cells, absence of connections. Internal vesicles absent. Hyphae, other than arbuscules, mostly > 5µm. External vesicles with knobs, on coiled hyphae .....Gi. calospora.

Hyphae rarely looped in epidermis, H connections may be present. Internal vesicles may be present. Hyphae of various sizes. External vesicles globose when present, on straight subtending hyphae III

III External vesicles common, globose, about 20µm diam., dark stained on subtending

hyphae about 1.5 - 3µm diam IV

External vesicles not common, larger if present, of variable staining V

IV Internal vesicles thin walled, up to 100µm long with an ovoid to irregular shape, sometimes lobed. Hyphae in root are not parallel, poorly stained c.f. arbuscules and vesicles ..... A. laevis

Internal vesicles thin walled, globose, 20 - 30µm diam. Internal hyphae irregular, 3 - 5µm diam. and stain darkly .....E. infrequens

V Internal vesicles infrequent, usually ovoid, thick walled when mature, to 100µm long. Internal hyphae mostly parallel .....Gl. albidum

Internal vesicles usually absent, hyphae can be darkly or lightly stained, of variable diam., hyphae may be parallel, loops may be present .....rest

Where VAM infections overlapped and where ectomycorrhizas were also present, identification of the VAM fungi was difficult and frequently only made to FE/CE. Where infections were older than about 28 days, overlapping colonies were common.

Ectomycorrhizas.

Ectomycorrhizal fungi were placed into five groups based on hyphal characters. Ascomycetes were the most commonly observed fungi. They usually had broad, hyaline, thin-walled hyphae with simple septa and an absence of clamp connections. There were three groups of ascomycetes. FM Asco I did not stain with Trypan Blue, though faint stain was occassionally left in the grooves between hyphae of the sheath of the ectomycorrhiza. FM Asco II became stained with Trypan Blue and there was always a dark blue deposit between hyphae of the sheath of the ectomycorrhiza. While these two groups were separated on staining characteristics, the morphology of infection was usually different as well. Hyphae of FM Asco I had irregularly shaped cells in the sheath, usually with an angular appearance (Fig. 11) whereas hyphae of FM Asco II were usually more rounded, with a more flowing appearance (Fig. 12).

Fig. 11. Surface of ectomycorrhiza formed by FM Asco I on Podotheca angustifolia. 1cm = 65um (stained in Trypan Blue)

Fig. 12. Surface of ectomycorrhiza formed by FM Asco II on Podotheca angustifolia. 1cm = 65um (stained in Trypan Blue)

Fig. 13. Surface of ectomycorrhiza formed by FM Asco III on Podotheca angustifolia. 1cm = 65um (Stained in Trypan Blue)

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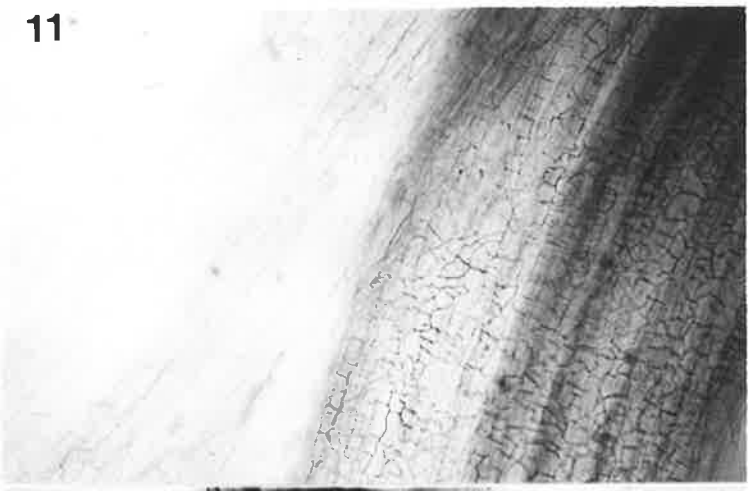

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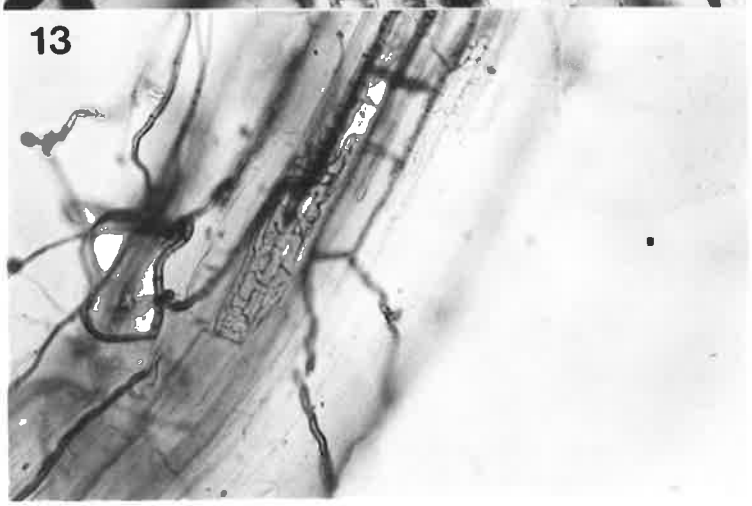


FM Asco III (Fig. 13) had dark brown hyphae and was probably Cenococcum. There was an aseptate, endogonaceous fungus present which formed ectomycorrhizas and this was presumed to be a species of Endogone, though zygospores were never observed. All basidiomycetes were grouped together. The commonly observed members had fine hyphae (1.5 - 3µm diameter) that stained palely in Trypan Blue. Clamp connections were always present on attached hyphae though not always present in mantles of ectomycorrhizas.

Not all ectomycorrhizas formed by ascomycetes could be placed with certainty into the groups FM Asco I, II or III. In a few mycorrhizas an intergrade between FM Asco I and II was observed. In such cases, the staining of hyphae away from the root was the predominant character chosen. Hyphae that stained well were considered to be FM Asco II, those that did not, FM Asco I.

Formation of Mycorrhizas

The growth cycle of annual and perennial plants differ in that roots of annuals usually commence growth near or at the soil surface after seed germination. Nutrient reserves of the seed may be small and the timing of germination may be critical for the survival of a species at the park. Reemergence of roots of perennials, on the other hand, is probably not as critical as nutrient reserves are comparativly large and conditions of growth below the soil surface not as variable as at the surface. Thus growth of and formation of mycorrhizas on annuals and perennials were examined separately.

Annual plants.

The seasonality of growth of annuals suggests that soil water content and temperature influence germination of seed. In 1983, opening rains fell on 1st March (Appendix 2) and follow-up rains occurred through March. In subsequent years either less rain fell initially or there were later follow-up falls of rain. In the field, observations of the emergence of seedlings were made about every seven days in 1983, at five day intervals in 1985 and 1986, and discontinuously in 1984.

After the opening rains of autumn, the first seedlings observed were Brassica tournefortii five days after the first fall of rain of greater than 15 mm, followed by Calandrinia volubilis and Crassula colourata within a further five days. All three species are nonmycorrhizal though vesicles were observed in some B. tournefortii. In 1983 and 1986, the first potentially mycorrhizal species were observed after 15 days. Plant species found then included Helipterum pygmaeum, Millotia tenuifolia, Plantago drummondii, Podolepis tepperi, Podotheca angustifolia and Wahlenbergia communis. In 1985, after the initial flush of plants that form mycorrhizas, no further potentially mycorrhizal seedlings were observed untill after the second period of rainfall (April 14/15, Appendix 2).

There was no apparent reason for the earlier emergence of non-mycorrhizal species. Seedlings of these species continued to emerge during the early part of the season. All seedlings of species known to form mycorrhizas collected from the field were found with mycorrhizas when their roots were longer than about 2cm. In most cases, the seedlings would have had two cotyledons with true leaves just emerging and would have been less than 5 days old. Those known to form both VAM and ectomycorrhizas were usually observed with both types.

As natural germination of annuals was usually irregular and the spacing sporadic, in January, 1986, 200 seeds of P. angustifolia were sown on a grid pattern in an open area of soil. The emergence of seedlings was monitored after the first rains in April, 1986. Counts of seedlings were made at five day intervals for 30 days (Fig. 14). Most seeds that germinated, did so within 15 days. Further germination would have probably depended on further rain. Except for those seedlings harvested, all seedlings survived to 30 days. Seedlings were collected from the field at five day intervals, five seedlings per collection. All plants were found with both CE and ectomycorrhizas. No seedlings were without mycorrhizas. All ectomycorrhizas were formed by ascomycetes (FM Asco I and II) except one plant from the harvest at 30 days, which had an ectomycorrhiza formed by a basidiomycete. FE was observed after 20 days on some seedlings.

It appeared that germination of seed in the field depended upon soil moisture. However, as soil temperature may have fluctuated significantly during early autumn,

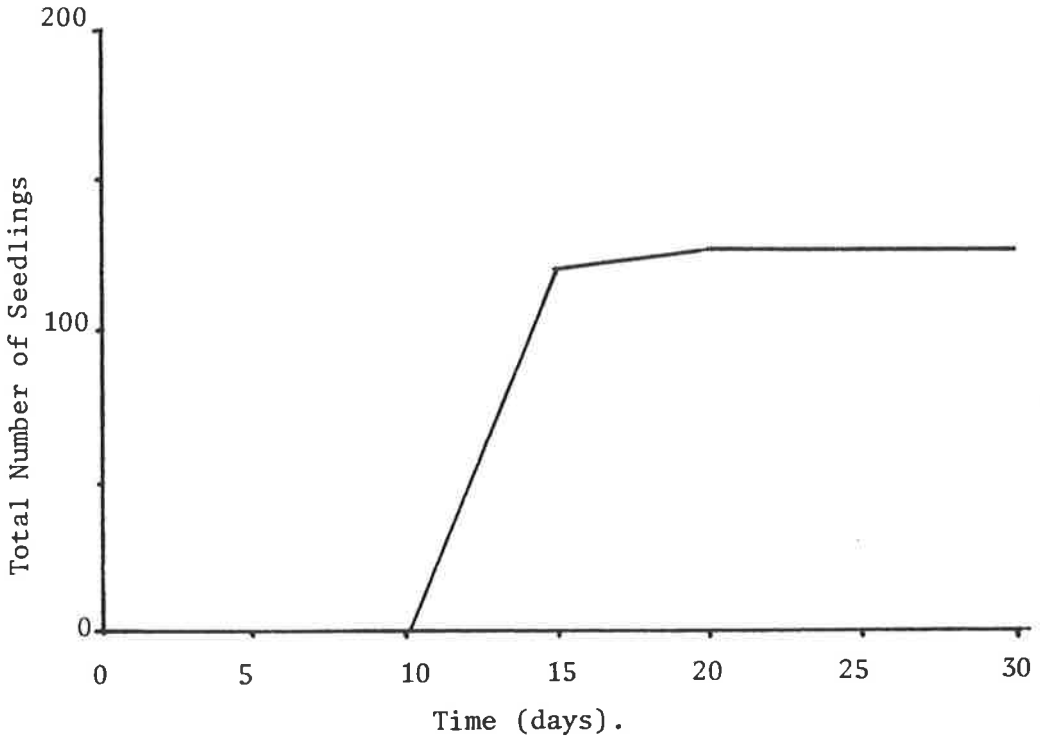


Fig. 14. Number of seedlings of Podotheca angustifolia emerged from 200 sown seeds after the first rains in April, 1986.

the importance of both soil water content and soil temperature appeared to be important for the germination and emergence of plants that form mycorrhizas. It would also seem that propagules of mycorrhizal fungi germinate earlier than the seedlings that they infect or that they germinate very rapidly in response to the emergent roots.

Perennial plants.

Commencement of regeneration of roots of the perennial species Melaleuca uncinata was examined after the opening rains of 1985 and 1986. M. uncinata was chosen as there are areas within the park where this species is the only perennial plant, so that all perennial roots collected belong to the species. As well, both VAM and ectomycorrhizas occur on M. uncinata and patterns of infection can be compared with P. angustifolia.

In both years four sites within 10m of each other were selected within a patch of M. uncinata. The closest tree or shrub of another species was more than 10m away from either site. At intervals of five days after the opening rains, a block of soil approximately 18cm^3 was collected from each site, each successive collection being from one face of the previous collection. Because random collections of soil from adjacent sites had soil water contents of less than one percent 10 - 15 days after rain, from the second soil collection in 1985 and at all collections of soil in 1986, about 6mm of deionised water was sprinkled over each collection area on each occasion after the removal of the block, in an attempt to maintain soil water content and thus root growth.

The blocks were returned to the laboratory. Soil water content was measured in each block of soil as a guide to the amount of water available to the plant. All roots were washed from each block of soil. All fresh roots, recognised as cream coloured extensions of the older brown or black roots, from each block were examined under a dissecting microscope then removed with 1 - 3cm of older root attached, cleared, stained and examined for mycorrhizas.

Soil temperatures were also measured at each collection of soil, adjacent to the sampling points using a thermometer with a bimetallic probe. Measurements were taken

at 0, 5 and 20cm down the profile. Three sites for measuring soil temperature were under litter and three sites were bare. All spot measurements were made at 11am Central Standard Time.

Root growth of M. uncinata commenced five days after the opening rains in 1985 and after 10 days in 1986. Long roots were usually observed to extend first though fewer than 5% of the existing long roots regrew initially. There was 1 - 2cm of fresh growth of a long root before some adjacent lateral roots broke through their ectomycorrhizas. Five to ten days later, when long roots had reached about 3 - 4cm, many lateral roots had emerged from them leaving 2 - 3cm of long root without laterals most of the time. While soil water content remained above about 3%, root growth continued, regardless of the temperatures recorded close by (Fig 15). Cessation of root growth was at soil water contents above permanent wilting point in the root zone (Fig. 16).

Fresh root growth was considered to have ceased when the root mass was found with only brown roots, after white roots had been previously observed. When roots were black grading to white on the tips, interpretation was difficult, though slow growth was considered likely. Certainly, the addition of water to the collection points increased the amount of root extension and the length of time over which extension was observed, when compared to roots collected from adjacent unwatered sites.

Even with the addition of extra water, soil moisture showed a large variation both over time and from point to point. The variation seemed to be random (Fig. 16) except in 1985 when reduction of soil moisture coincided with high soil temperatures at the surface (Fig. 15). Interpretations should be cautious, though, as data are so incomplete. It is likely that roots were absorbing water (either directly or through their mycorrhizas) as soil water levels dropped. All collection points chosen had an apparent concentration of roots from about 5 - 30cm, and that root zone seemed to dry out faster (to touch) compared to zones with fewer roots lower in the soil profile.

Roots of M. uncinata were examined for mycorrhizas. While there was variation between samples, a general pattern emerged in 1985 and was repeated in 1986. Presence of fresh CE and FM Asco I and II were observed first on the recent growth of

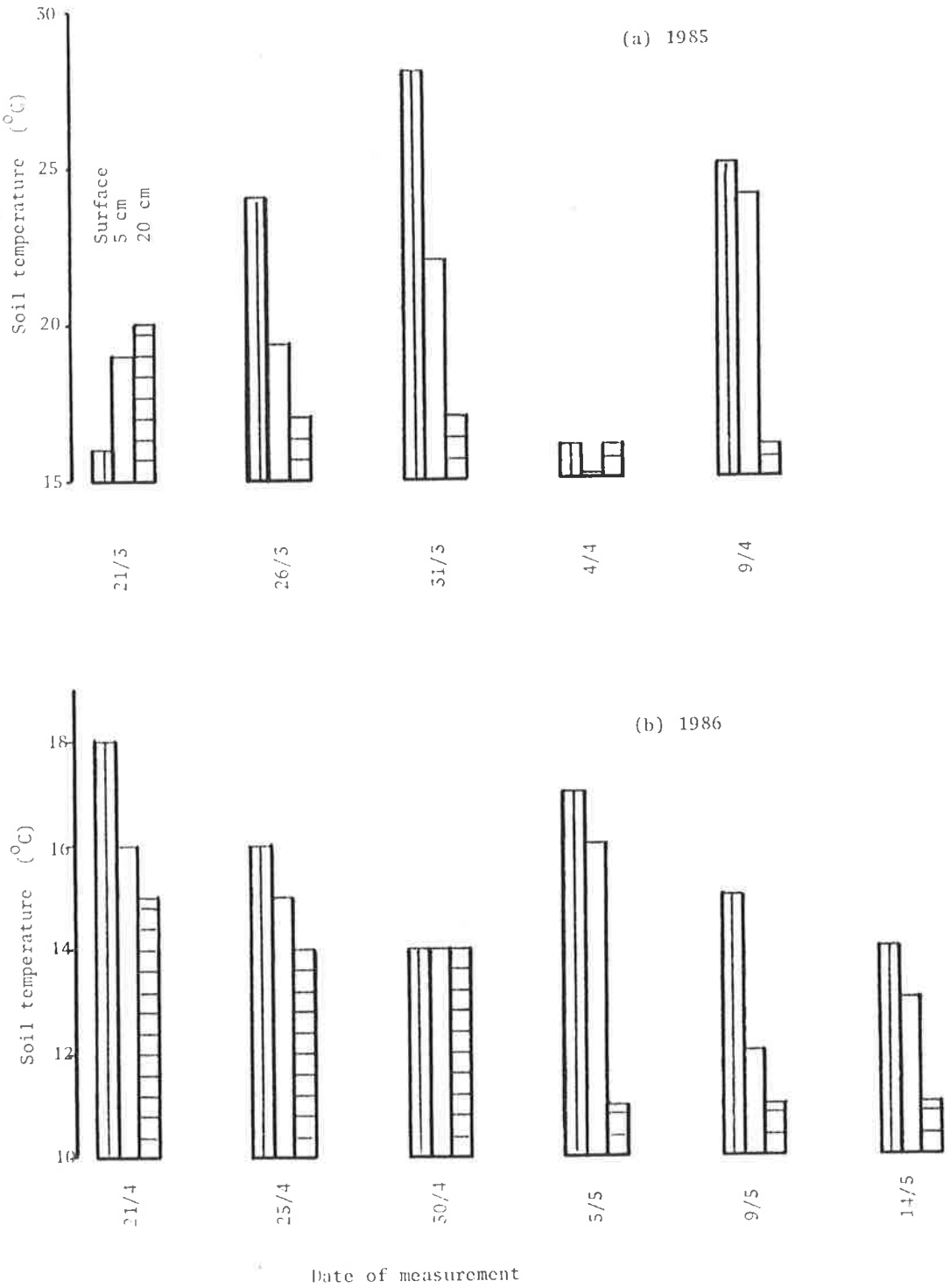


Fig. 15. Soil temperatures of a profile adjacent to the collections of roots of Melaleuca uncinata for observation of root growth and presence of mycorrhizas. Note difference of scales.

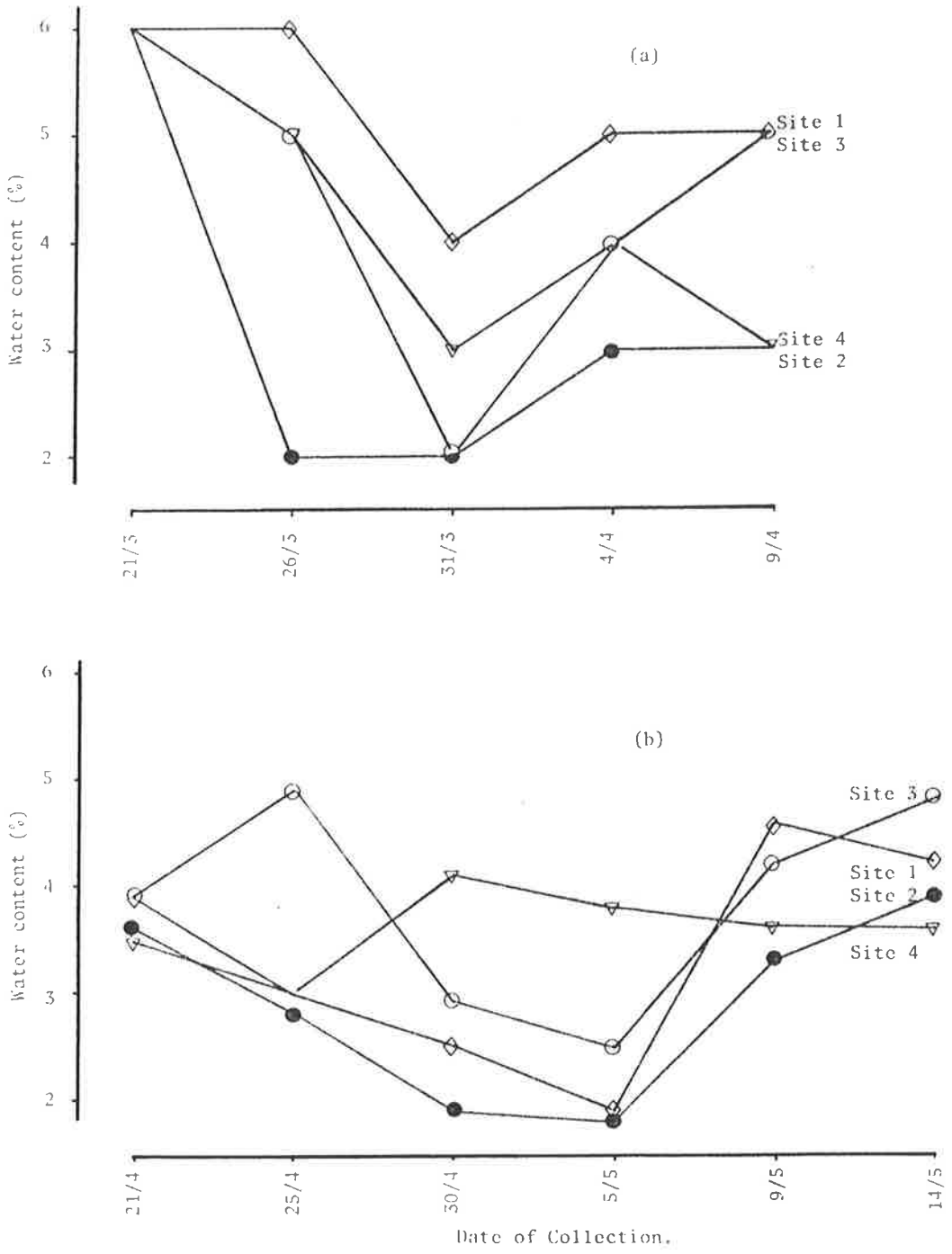


Fig. 16. Soil water content of soil collected for the observation of root growth of and presence of mycorrhizas on Melaleuca uncinata.

long roots. Hyphae of CE and FM Asco I could be traced into the soil in all cases, though this is not to say that the hyphae did not arise from a mycorrhiza elsewhere on the root system. FM Asco II came from old ectomycorrhizas or grew from the soil. In separate cases, FM Asco II was observed to grow from a rhizomorph and from sclerotia-like structures, adjacent to the root. Initial infections of mycorrhizal fungi occurred five days after the first observation of root extension. FM Asco III first occurred I5 days after first root extension, growing from old ectomycorrhizas. Endogone was only observed once in 1985, growing from an old ectomycorrhiza, some 15 days after initial root extension. Basidiomycetes were observed on fresh roots on the last collections of each year, always growing from old ectomycorrhizas. Observations ceased when ectomycorrhizas started to obscure VAM making interpretation difficult. At the end of the growing season basidiomycetes were always the most common fungi, suggesting that there was a succession (Mason et at, 1983) of ectomycorrhizal fungi on the roots of M. uncinata over the season.

The most common VAM fungus observed was Gi. calospora, with two other unidentified VAM fungi also present. No FE was observed. Initial mycorrhizal infection occurred on the long roots, with CE being in the roots and hyphae of ascomycetes being found on the roots in the junction of epidermal cells. Ectomycorrhizas usually formed on short roots where CE were only observed when ectomycorrhizas were absent. Wefts of hyphae from basidiomycetes often covered the long roots without infection.

Discussion

(Table 9)

The field data showed that VAM fungi with medium sized spores were found near the soil surface (A. laevis, E. infrequens and Gl. albidum) and the fungus with larger spores (Gi. calospora) was lower in the soil profile. Gi. calospora was rare in the upper 10cm of soil and the species with medium spores were rare at depth. In the few surveys elsewhere of vertical distribution of spores of VAM fungi, there has been a maximum density of spores near the soil surface with a decrease in spore numbers and the number of species found with depth (Sutton & Barrow, 1972; Smith, 1978; Zajicek, Daniels Hetrick & Owensby, 1986). In all cases, the species found at depth were also found near the soil surface, and they were also the most common spore type found.

An explanation for the absence of Gi. calospora near the soil surface is that the fungus does not survive the wet/dry cycles over summer in the absence of plant growth (Chapter 5). Further, Gi. calospora does not seem to regrow into the upper soil layer. Spores of the fungus are not found in the surface 10cm at the end of winter. Roots of annual plants collected from the upper 10cm of soil, late in the season, were not infected with Gi. calospora. The roots of annual plants appear to be concentrated in the top 20cm of soil. On the other hand, roots of perennial plants, which are mostly found from 5 - 30cm below the soil surface, have been found with infections of Gi. calospora. That Gi. calospora does not grow into the surface soils suggests that competition from existing fungi prevents such regrowth. However, competition cannot explain the absence of the surface fungi at depth.

There is no evidence to suggest that the isolate of Gi. calospora found at the park varies from isolates found elsewhere. Morphology of spores and infection units do not differ from published descriptions (Abbott & Robson, 1978; Hall & Abbott, 1984). In agricutural soils, the spores of the fungus are found near the surface (Tommerup, 1983), so presumeably can compete successfully with other fungi under certain conditions.

Soil temperature and water content of soil appeared to affect the germination of fungi and the establishment of mycorrhizas. Further, formation of mycorrhizas differed between annuals and perennials suggesting competitive interactions between plants and fungi. These factors were examined experimentally.

Chapter 5

EXPERIMENTAL STUDIES

From the field studies, it was apparent that a number of factors influenced the formation of mycorrhizas. Study of germination of seed and reemergence of roots of perennial species and the formation of mycorrhizas on the roots suggested that germination of fungal propagules was independent of plants and possibly influenced by soil temperature and soil water content. The progress of mycorrhizal infection also appeared to differ in different plant species suggesting that competitive interactions might occur.

Further, the genus Thysanotus was observed with a fungal association that did not fit readily into any of the standard groups of mycorrhizas. The nature and morphology of the association were examined in more detail.

Methodological Problems

As VAM fungi cannot yet be grown successfully in agar culture, they are normally grown singly in association with an appropriate host in pot culture. As natural soils contain propagules of VAM and other mycorrhizal fungi, the soil is usually autoclaved or otherwise treated to remove mycorrhizal fungi. Autoclaving some soils may lead to subsequent problems of plant growth.

Effect of Treatment of Soil

To examine the response of mycorrhizal fungi to environmental factors it is important that the experimental soil be similar in physical and chemical properties to the soil of origin of the experimental fungi (Daniels Hetrick, 1984). To obtain soil free of fungi, soils from which the fungi have been obtained are often treated with either chemicals, heat or gamma irradiation. Autoclaving or gamma irradiation are the

preferred methods as all microbes can be killed. Penetration of chemicals into bulk soils is not always complete thus the treatment is unreliable (Baker, 1970). Equipment to autoclave soil was available. Treatment by gamma irradiation was time consuming and expensive as soil had to be sent to Melbourne for treatment. Treatment by aerated steam (30 - 45 minutes at 60 - 70^{o}) does not always kill all ascomycetes and fine hyphaed VAM fungi (Warcup, 1981). Treatment of soil in a microwave oven (Ferris, 1984) was used to heat soil differentially but was only suitable for small quantities. Hence autoclaving was the preferred treatment.

A single seedling of either T. subterraneum, Plantago drummondii or Solanum opacum was grown for six weeks in autoclaved bulk soil collected from 0 - 10cm deep, after inoculation with spores of Glomus albidum, Gigaspora calospora or Acaulospora laevis, or 0.5g fresh weight of VA mycorrhizal root pieces of Trifolium subterraneum that had been grown in untreated Ferries McDonald soil for eight weeks. Plants were grown in untreated soil for comparison. At harvest roots were cleared, stained and examined microscopically for VAM. No inoculated plants were found with VAM though plants grown in untreated soil became mycorrhizal.

To determine if heat treatment of soil altered available minerals, mineral analysis of steam-treated, autoclaved and untreated soil by Adelaide and Wallaroo Fertilizers Ltd (Table 13) was done showed that heat treatment increased the amount of extractable sulphate, manganese and decreased the level of iron in the soil and that soil from the upper profile had more nutrients than soil from lower in the profile. It (EDTA extraction) These results suggested that high available manganese may have been responsible for the lack of formation of VAM in the previous experiment. Samuel (1926) noted that roots of oats grown in soil with low levels of available Mn had more VAM than oats from Mn sufficient soils. Menge et al (1982) and Ojala et al (1983) have found levels of P, Zn and Mn extracted from several soils could also be used to predict the mycorrhizal dependancy of Troyer Citrange which was more dependant on mycorrhizas in soils with low levels of P, Mn and Zn. These results suggest a relationship between levels of available Mn and VAM. Hepper and Smith (1976) found that as little as 13.6 µg/g Mn (as $MnSO_4$) reduced the percentage of spores of Glomus mosseae that germinated on agar and also inhibited

Table 13. Mineral analysis of bulk soil samples from Ferries McDonald Conservation Park collected from 0 - 10 or 20 - 30cm down the soil profile treated by aerated steam, autoclaving or left untreated.

Mineral analysis		Depth					
		0 - 10cm			20 - 30cm		
		Soil treatment			Soil treatment		
		Nil	Auto	Steam	Nil	Auto	Steam
Org. Carbon	%	1.6	1.6	1.5	0.2	0.2	0.2
Nitrate	$mg.kg^{-1}$	3.0	3.4	3.0	0.6	0.8	0.4
Sulphate	"	4	8	5	2	2	2
Phosphorus	"	6	6	6	1	1	2
Chloride	"	5	5	10	10	10	25
Copper	"	0.1	0.1	0.1	0.1	0.1	0.2
Zinc	"	0.4	0.4	0.4	0.1	0.1	0.1
Manganese	"	4	9	5	1	1	1
Iron	"	20	16	17	31	25	29
Potassium	$meq.100g^{-1}$	0.12	0.13	0.14	0.06	0.05	0.06
Calcium	"	3.7	3.9	3.8	0.9	0.8	0.9
Magnesium	"	0.9	0.9	0.9	0.3	0.2	0.2
Sodium	"	0.9	0.9	0.9	0.3	0.2	0.2

growth from pregerminated spores.

Effect of Manganese on Plant Growth and Mycorrhizas

To examine further the effect of high available Mn on mycorrhiza formation, plants of S. opacum were grown for six weeks in soil that had been treated by autoclaving (120^{o}C for one hour), aerated steam (30 minutes at 60^{o}C), gamma irradiation (5 MRad) or left untreated. Some of the autoclaved soil had a soil filtrate, obtained from mixing 200g of moist, untreated soil with 300ml of sterile deionised water and filtering through an 8µm Millipore filter, added to it. Twenty ml of filtrate was added to each pot. As well, a bulk soil that had been autoclaved three months earlier and subsequently stored dry, was included. At harvest, the roots were examined for VAM and the concentration of Mn in the shoot tissue was determined by digesting shoots in nitric/perchloric acids, and measuring the concentration of Mn in the digests using an atomic absorption spectrophotometer with background correction.

The data in Table 14 show that mycorrhizas were only found in plants grown in untreated soil and that levels of Mn were higher in the shoots of plants grown in treated soil. Even after three months storage, significantly higher levels of Mn were available to the plant from autoclaved soil. Further, adding a soil extract did not greatly lower the amount of available Mn.

Manganese may inhibit the formation of VAM by acting on the plant or on the fungus. If Mn acts on the plant, there may be separate effects on plant growth and formation of mycorrhizas. If Mn acts on the fungus, there may be inhibition of germination of fungal propagules or growth of hyphae through the soil. The effect of Mn on plant growth with and without VAM was examined.

To obtain an untreated soil free of VAM fungi was not considered possible, so to examine the effects of Mn on plant growth in the virtual absence of VAM, a soil with low levels of natural inoculum was used. Bulk surface soil was sieved and moistened with either deionised water or solutions of Mn salts as $MnSO_4$ or $MnCl_2$ in deionised water

Table 14. Concentration of Manganese in shoots and the presence of VAM in roots of Solanum opacum grown for six weeks in treated soil or after various sterilization treatments. (Mean of three replicates).

Soil treatment	Concentration of Mn (µg/g)	Presence of VAM
Nil	14.9	+
Gamma irradiated	109	-
Aerated steam	49	-
Autoclaved three months prior to use	54	-
Autoclaved immediately prior to use	131	-
Autoclaved immediately prior to use with a soil filtrate added	105	-

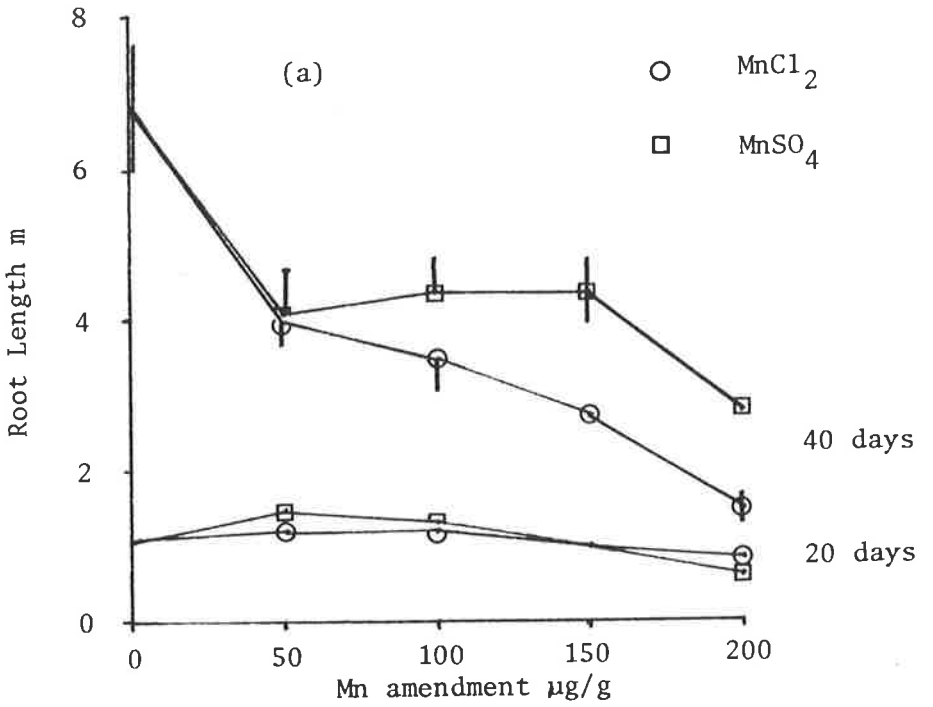
such that the soil was amended with either 0, 50, 100 or 200µg/g Mn. Seedlings of S. opacum were transplanted into pots, four seedlings per pot for harvesting at 20 days and one seedling per pot for later harvests, with four replicate pots per treatment. At 20 and 40 days the plants were harvested, the dry weight of shoots, total root length, length of root infected with total VAM, CE and FE determined and the percent root length infected with total VAM, CE and FE calculated.

No plants at 20 days and about half the plants at 40 days had VAM. Less than 1% of root length was infected in all cases. Growth of S. opacum was not affected significantly by Mn at 20 days, however, by 40 days there was a significant inhibition of dry weight of shoots and length of root of all plants grown in soil amended with Mn (Fig 17), the effect being most noticeable in the $MnCl_2$ treatment.

Manganese reduced growth of practically non-mycorrhizal plants. In determining the effect of Mn on mycorrhizal plants it was necessary to examine the effect of increased Mn in the soil on the growth and infection parameters over a range of Mn that did not affect plant growth in the absence of mycorrhizas. Thus in a further experiment, seedlings were grown for 20, 40 or 60 days in soil with adequate levels of mycorrhizal inoculum, which had been amended with 75, 100, 125 or 150µg/g Mn as $MnSO_4$. At harvest length of root, shoot dry weight, length of total VAM, CE and FE were determined and the percentage root length infected with total VAM, CE and FE calculated. The concentration of Mn in the shoot tissue was also measured.

Growth of mycorrhizal S. opacum was significantly greater at 150µg/g Mn than at lower levels (Fig 18). Percent root length infected with mycorrhiza declined with increased levels of soil Mn (Fig 19) at 20 and 40 days though increased at 60 days. At 20 days there was a significant negative correlation between Mn applied and the percent root length infected with VAM ($r = -0.555$, $p=0.013$), with %CE ($r = -0.531$, $p = 0.017$) and with % FE ($r = -0.508$, $p=0.022$). Concentration of Mn in the shoot tissue (Fig 20) declined over time, though from 40 to 60 days the decline was not significant within each treatment. At all harvests Mn added to the soil correlated with concentration of Mn in the shoot tissue ($p < 0.001$) but there was no significant

Fig. 17. Growth of non-mycorrhizal Solanum opacum after 20 or 40 days in soil amended with 0, 50, 100, 150 or 200 ug/g Mn as $MnCl_2$ or $MnSO_4$. (a) Root length; (b) Shoot dry weight; mean * SEM of four reps.



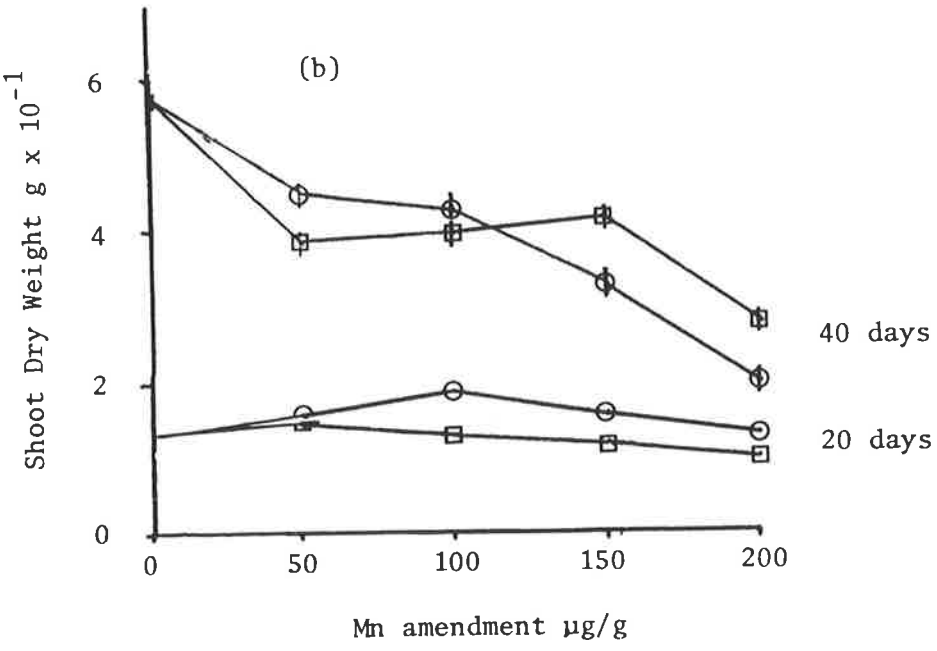
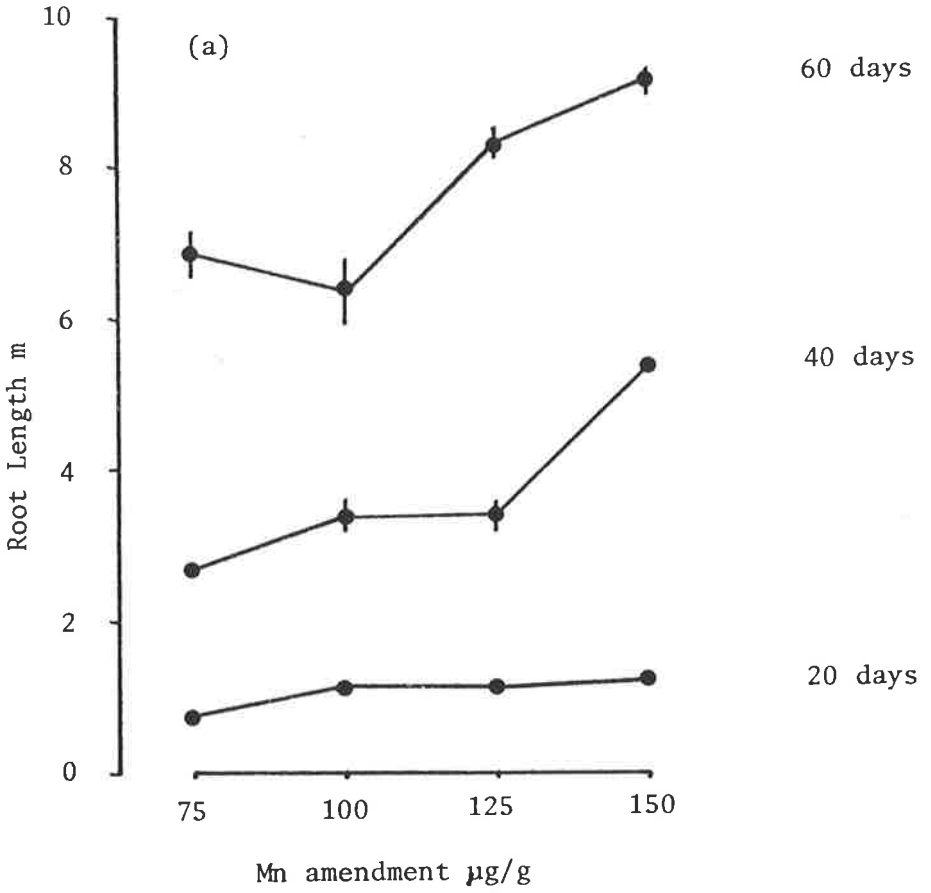


Fig. 18. (a) Length of root and (b) shoot dry weight of mycorrhizal Solanum opacum after 20, 40 or 60 days in soil amended with 75, 100, 125 or 150µg/g Mn as $MnSO_4$. Mean ± SEM of four reps.



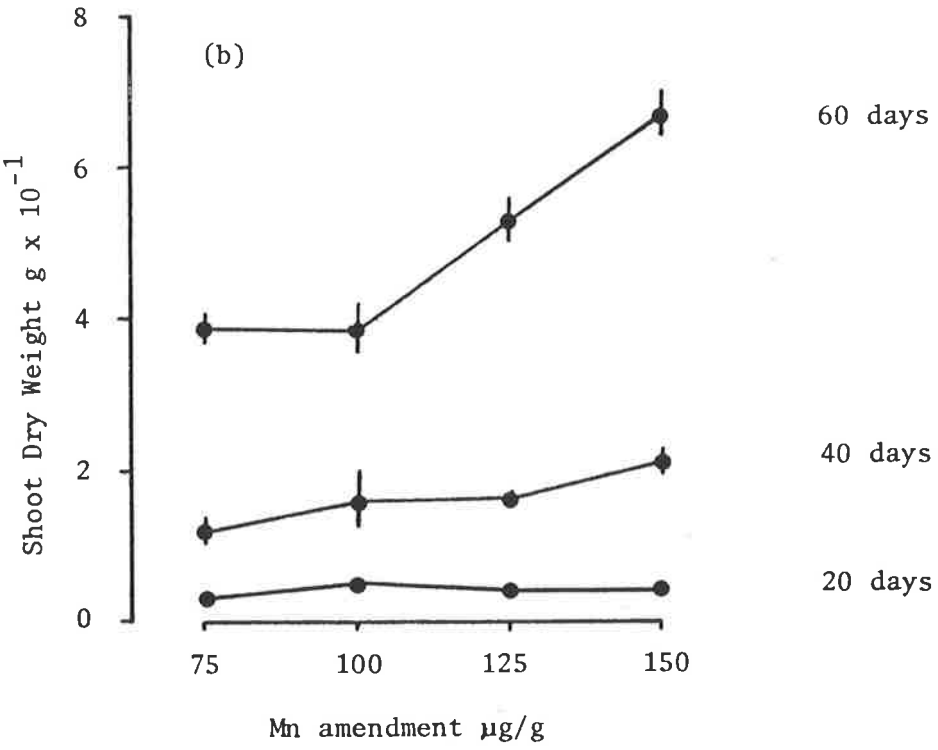
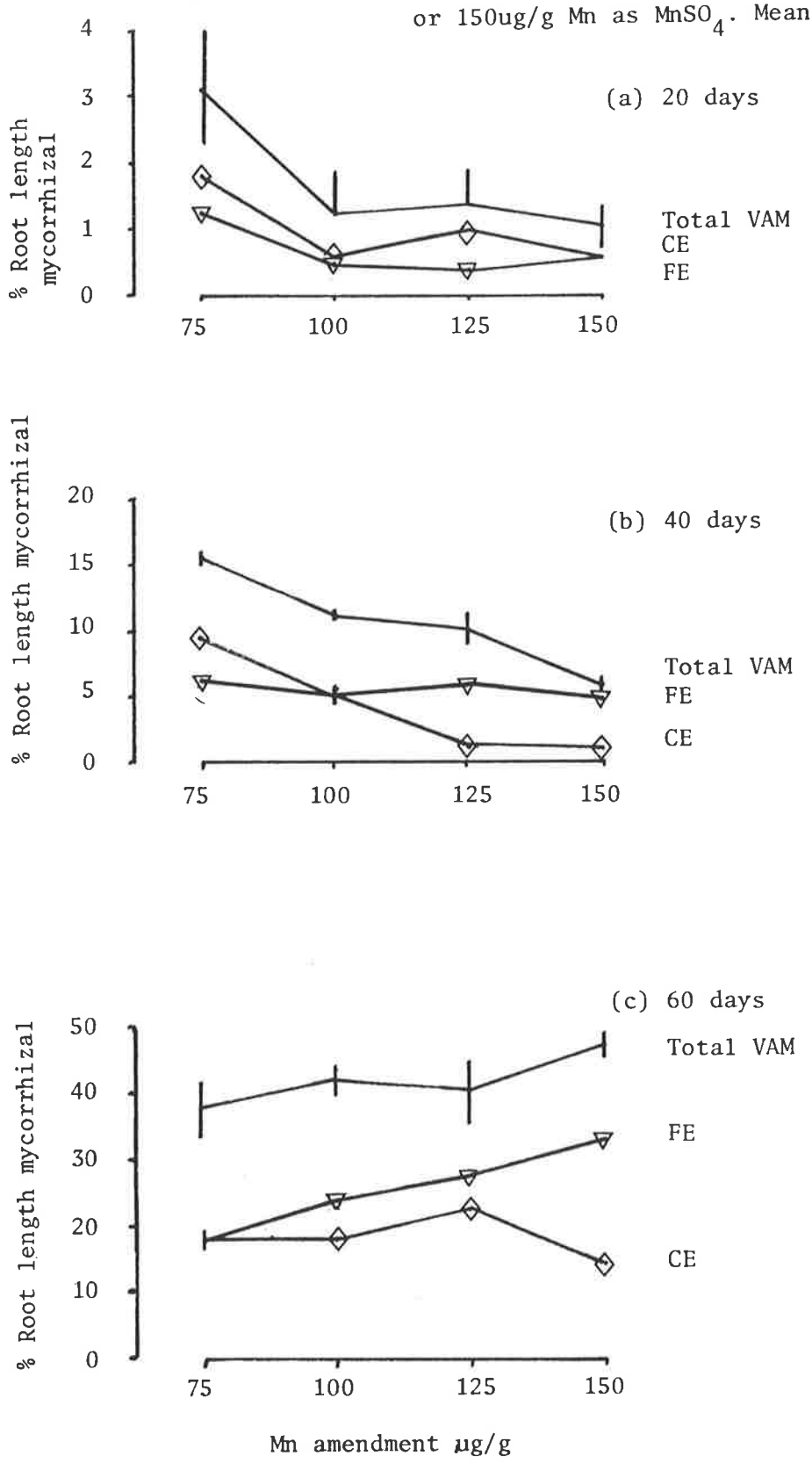


Fig. 19. Percent of root length of Solanum opacum infected with total vesicular-arbuscular mycorrhiza, coarse endophyte or fine endophyte after (a) 20 days, (b) 40 days or (c) 60 days in soil amended with 75, 100, 125 or 150ug/g Mn as $MnSO_4$. Mean * SEM of four reps.



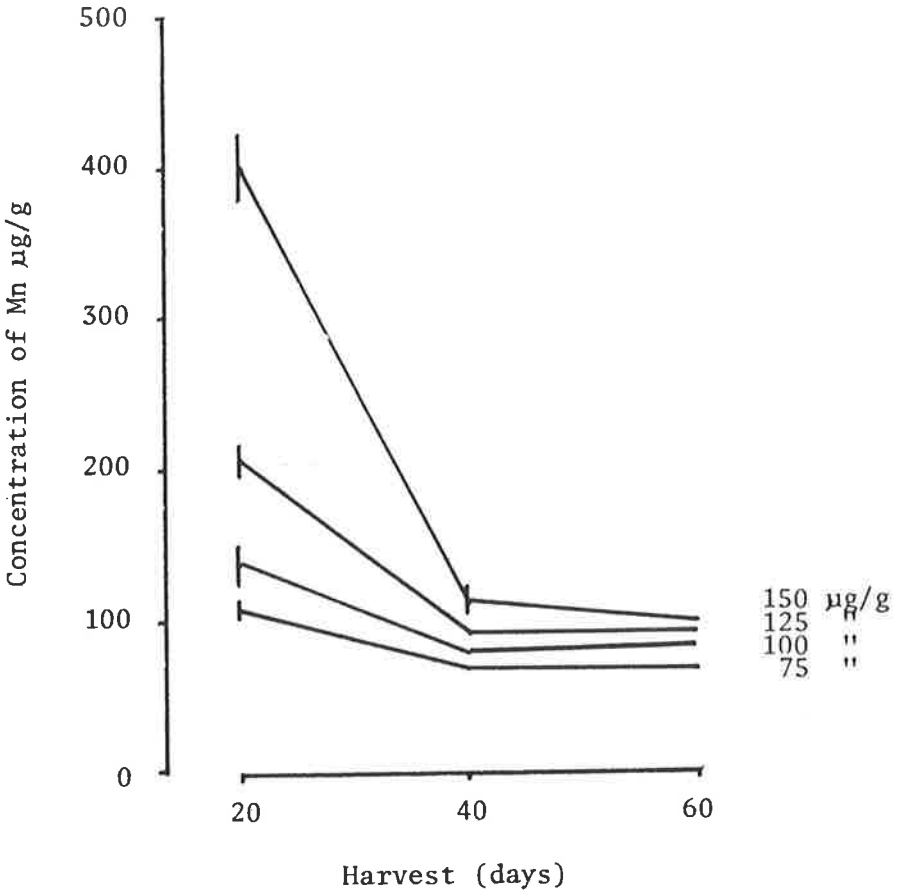


Fig. 20. Concentration of Mn in shoots of mycorrhizal Solanum opacum grown for 20, 40 or 60 days in soil amended with 75, 100, 125 or 150µg/g Mn as $MnSO_4$. Mean • SEM of four reps.

correlation between concentration of Mn in the shoot tissue and % total VAM, % CE or % FE. These data suggest that Mn in the soil acted directly on the fungal propagules in the soil and either inhibited germination and /or inhibited the spread of and infection of roots by the germinated fungi.

The propagules of fungi that initiated formation of VAM could have been either spores or hyphae in senescent root pieces. Thus it was necessary to examine the germination of each type of propagule and the effect of Mn on the rate of germination. The number of spores of VAM fungi in the bulk soil was low, there being between 0 and 30 spores per 100g of dry soil of G. albidum, the most common fungus. Spores of G. albidum were wet sieved from the soil and were either rinsed directly, three times in sterile deionised water or after being placed in 1% sodium hypochlorite for 30 seconds. Spores were then either placed on water agar, 20 spores per plate or sandwiched between two Millipore filters (8μm pore size; Tommerup, 1984), 25 spores per sandwich and buried in moistened soil. Spores on agar were examined for germination thrice weekly for 42 days. Millipore filter sandwiches were harvested at 7, 21 or 42 days with three replicates of each treatment. All spores in Millipore filter sandwiches were examined for germination at harvest after staining overnight in Trypan Blue.

No spores had germinated on agar by 42 days. Of the spores placed in soil between Millipore filters, none had germinated by 21 days. At 42 days, three, four and 10 out of 25 non-sterile spores had hyphae longer that 100μm emerging from them. Given the low spore densities and the delay in spore germination after imbibation, spores clearly did not contribute significantly to the early stages of root infection. Thus it was necessary to examine the effect of Mn on the emergence of hyphae from root pieces.

Roots of T. subterraneum were removed from dried pot cultures of G. albidum and FE and tested for emergence of hyphae. Fifty segments of root about 1cm long were placed in Millipore filter sandwiches between pieces of filter paper. The edges of the filter paper were folded to retain the root segments. The units were then placed in moistened soil that had been amended with Mn as $MnCl_2$ at either 0, 100 or 200μg/g Mn. Root segments were examined for exit points of VAM fungi after staining overnight

in Trypan Blue followed by heating for five minutes. Harvests were at 14 and 21 days. There were four replicates of each treatment. VAM fungi had emerged from root pieces by 14 days and Mn reduced the number of hyphae that emerged (Table 15). From these results, it is probable that Mn had a role in inhibiting germination of propagules of VAM fungi. To reduce the effects of available Mn in the experimental soil, either the level of Mn or the availability of Mn needed to be reduced.

The Experimental Soil

Soil collected from 20 - 30cm down the profile had lower concentrations of both Mn and organic carbon (Table 13) than the surface soil. Organic matter is the habitat of a large proportion of the viable soil microflora, in the absence of roots (Fuller, 1974). As the oxidation state of Mn is mainly determined by soil microbes and Mn is closely associated with soil microbes, the relationship between Mn and soil organic matter was examined by determining their coincidence in soil from the park under a range of conditions that may affect the level of available Mn.

There were six soil treatments. A bulk soil collected from 0 - 10cm deep was either left untreated, heated dry for two hours to mimic heating of soil in the field, autoclaved for one hour or had 100µg/g Mn as $MnSO_4$ mixed throughout dry soil. Soil was also collected in summer from a site with a bare surface and from a litter covered site. Soil temperatures on the day previous had reached 65 - 70^{o}C at an adjacent bare site and about 35^{o}C at a litter covered site (Appendix 3). There were five replicates of each treatment. All soils were sieved through a 2mm pore size sieve and then measured for extractable Mn by members of the Department of Agriculture Soil Analysis Laboratory, using DTPA at pH 7.3. The results in Table 16 show that dry heating did not affect levels of soil Mn and that autoclaving and addition of Mn increased extractable Mn significantly. Percent organic carbon was also measured in the soils (Table 16). There was a significant correlation between Mn and % organic carbon for the soil samples collected in January (bare surface: $r = 0.9$, $p < 0.05$; under litter: $r = 0.8$, $p < 0.05$

Table 15. Number of hyphae of vesicular-arbuscular mycorrhizal fungi growing from segments of dried mycorrhizal roots observed at 14 or 21 days after burial between Millipore filters in soil amended with 0, 100 or 200µg/g Mn as $MnCl_2$. Mean ± SEM of four reps.

Amendment µg/g	0		100		200	
Harvest (days)	14	21	14	21	14	21
Fungus						
Glomus albidum	5.3±1.3	N.D.	0	0	0	0
Fine endophyte	7.0±2.6	15.3±8.3	0.5±0.5	0.5±0.5	0	0

N.D. - not determined, fungi from the soil invaded the space between the Millipore filters.

Table 16. Percent organic carbon and manganese extracted from soil treated with the natural heat of summer, dry heat, autoclaving, by adding 100μg/g Mn as $MnSO_4$ or left untreated. (Mean ± SEM of five replicates).

Soil Treatment	Mn extracted mg Kg^{-1}	% C
Bulk soil	0.98 ± 0.01	0.55 ± 0.05
Bulk soil heated to 70°C for 2 hrs	1.0 ± 0.01	0.56 ± 0.05
Bulk soil autoclaved	6.72 ± 0.15	0.55 ± 0.06
Bulk soil amended with Mn	27.4 ± 0.08	0.54 ± 0.06
Soil collected from a bare surface the day after the soil temperature reached 70°C	1.8 ± 0.7	0.64 ± 0.2
Soil collected from under litter where the soil temperature reached 35°C	3.2 ± 0.9	1.1 ± 0.1

using Spearman rank difference correlation). These data show that where soil treatment was required, soil with low levels of organic matter should be used to avoid significant increases in available Mn after soil treatment.

Soil from 15 - 30cm depth has fewer mineral nutrients and level of Mn is apparently unaffected by autoclaving (Table 13). Bulk collections of soil from 15 - 30cm deep were sieved and autoclaved. Seedlings of S. opacum were transplanted into pots of moistened soil over inoculum of 0.5g fresh mycorrhizal roots. All seedlings became mycorrhizal within four weeks. As well, storage for a period of time may decrease available Mn (Rovira & Bowen, 1966; Table 14). Standard bulk soil for all experiments where the medium had to be free of mycorrhizal fungi was obtained from 15 - 30 cm deep, sieved, autoclaved and then stored dry for at least six months before use.

Levels of extractable P in the bulk soil were low (1.0µg/g, Table 13) compared to the surface soil (6.0 µg/g). Plant growth is slow in soils deficient in available P and the total length of roots and thus mycorrhizas might be less than that obtained from plants grown in soil with more adequate levels of P. As pot cultures were to provide fungal inoculum for experiments, if more inoculum could be obtained from one plant, fewer plants in pot culture would be required. Also, if plant growth could be increased experimentally, then roots may have contacted more germinated fungal propagules and formed more mycorrhizas. Thus response curves of mycorrhizal fungi to phosphorus in soil were determined.

Bulk soil collected from the soil surface and containing natural inoculum and sieved but otherwise untreated was amended with either 0, 5, 10, 20, 40 or 80 µg/g P as Na_2HPO_4. Seedlings of P. drummondii were transplanted into pots of amended soil and harvested after four weeks. At harvest, shoot dry weight, root length, and length of VAM, CE and FE were measured and percent VAM, CE and FE calculated. It was found (Table 17) that length of VAM, %CE and %FE were reduced by added phosphate.

The effect of added phosphate on two ectomycorrhizal fungi and one VAM fungus was investigated. Two pots of Podotheca angustifolia were each inoculated with 0.5g of roots infected with either Peziza whitei, Laccaria ohiensis or Glomus albidum in autoclaved bulk soil amended with 0, 2, 4 or 8 µg/g P as Na_2HPO_4. In contrast to the first experiment, only in the unamended soil were hyphae growing into the soil from the pad of inoculum at 21 days. A further two pots of Trifolium subterraneum were inoculated with G. albidum in soil amended with 5µg/g P. After six weeks the plants were harvested and found to be mycorrhizal. Roots were then used as inoculum under single seedlings of P. drummondii in pots of autoclaved soil, two pots with and two pots without an amendment of 5µg/g P. At harvest after six weeks, all plants were non-mycorrhizal. Formation of mycorrhizas varied with phosphate added to the soil. Propagules of VAM fungi in untreated amended soil initiated mycorrhizas. However,

Table 17. Growth of shoots, roots and spread of VAM in Plantago drummondii grown in soil amended with phosphate. (Mean ± SEM of five replicates.)

Added P µg/g	Root Length cm	Shoot d.w. $gx10^{-1}$	Length of VAM cm	%CE	%FE
0	284 ± 49	2.9 ± 0.2	20 ± 3.5	3.4 ± 0.8	2.1 ± 0.3
5	257 ± 24	2.5 ± 0.2	5 ± 2.3	1.0 ± 0.6	0.8 ± 0.3
10	303 ± 38	3.2 ± 0.2	12 ± 2.5	1.0 ± 0.6	2.3 ± 0.9
20	250 ± 53	2.3 ± 0.5	2 ± 0.5	0.6 ± 0.2	0.3 ± 0.3
40	246 ± 48	4.9 ± 0.6	1 ± 0.4	0.4 ± 0.2	0
80	335 ± 53	4.6 ± 0.7	0	0	0

when fresh mycorrhizal roots were used as inoculum in autoclaved soil, infection was limited in the presence of P, particularly when the root inoculum had been grown in amended soil. Except for some initial experiments on germination of propagules of mycorrhizal fungi in natural soil, no P was added to experimental soils.

Initiation of Mycorrhizas

The initiation of mycorrhizas at the start of the growing season relies upon emerging roots from either seed or dormant roots meeting hyphae growing from propagules of mycorrhizal fungi. Germination of hyphae may be dependant upon or independant of emergence of roots. As several different groups of mycorrhizal fungi and roots of many species of plants are found in close proximity, there is likely to be competition between fungi for infection of roots with each fungus possibly having a different pattern of infection. With such alternatives possible, the initiation of mycorrhizas at the start of the growing season was investigated experimentally.

Germination of Seed of Annual Plants from the Park

As field observations indicated that soil temperature and soil water content influenced the rate of germination of seed, seed germination was examined in the laboratory. The rate of germination of seed on moistened sand was examined first. Temperatures in the growth room were from 16 to 22^{o}C. Rates of germination observed were slightly faster (Table 18)than in the field (Chapter 4). Seed was also germinated on blocks of soil from the park moistened to either 8 or 10% by weight with deionised water and placed in water baths. Lids of plastic petri dishes were placed on the pots to retain moisture within the containers. Germination of seed on the blocks (Table 19) was reduced by high soil temperature and of P. angustifolia and Pl. drummondii at low soil water content.

The effect of soil temperature and water content was examined further by observing the emergence of volunteer seedlings in an experiment to investigate infection of transplanted seedlings. In blocks of soil moistened to 8 or 10% set in water baths, non-target seedlings were counted and removed when first observed. From Table 20, it is apparent that germination of seedlings increases in soils with low temperatures and high water content. The identified seedlings removed from the

Table 18. Time to seedling emergence and percent germination of seed of four species placed on moistened sand in the growth room.

Plant species	Time to germination	Percent germination
Podotheca angustifolia	8 - 12 days	85*
Plantago drummondii	4 - 7	95*
Blennospora drummondii	9 - 12	80*
Helipterum pygmaeum	2 - 3	100+

* - 100 seeds tested, + - 50 seeds tested.

Table 19. Time to seedling emergence and percent germination of seed of four species placed on the surface of blocks of soil moistened to 8 or 10% water content and set in Wisconsin tanks at 16, 20 or 30°C.

Soil temp. °C	Soil water content (%)	*Podotheca angustifolia*	*Plantago drummondii*	*Blennospora drummondii*	*Helipterum pygmaeum*
		Percent germination of seed			
16	8	13[1]	70[1]	89[2]	89[2]
	10	53	97	100	100
20	8	0	0	11	0
	10	7	37	33	0
30	8	0	0	0	0
	10	0	3	0	0
		Time to emergence of seedlings (days)			
16	8	12-13	6-12	12-17	12-14
	10	10-12	6-9	13-15	12-14
20	8	-	-	18	-
	10	12	6-10	15-17	-
30	8	-	-	-	-
	10	-	7	-	-

1 - percent germination of 30 seeds

2 - of 9 seeds.

Table 20. Number of volunteer seedlings removed from three blocks of soil, each moistened to either 8 or 10% water content and placed in water baths set at either 16, 20 or 30°C, over four weeks.

		Number of seedlings		
		16°C	20°C	30°C
Water	8%	18	0	0
content	10	116	18	1

treatment with 10% water content were either Blennospora drummondii, Caladrinia volubilis, Crassula colorata, Helipterum pygmaeum, Millotia tenuifolia and P. angustifolia and from 8% water content, H. pygmaeum. Most seedlings were not identified, however, as they were so small when removed.

In the field, seed of annual mycorrhizal plants first germinate 10 - 15 days after the first significant rains of autumn each year. The rate of germination is influenced by both soil temperature and in most cases by soil water content.

Germination of Spores of VAM Fungi

The number of spores per 100g dry soil (7 - 13, Table 9, p35) was similar to the number of propagules of CE (2 - 10, Table 10, p36; 26, Table 24, p93; 12, Table 25, p 96; 13.3, Table 27, p106) in the surface soils from the field. If spores are the principle source of inoculum of VAM fungi in this soil, then germination of the spores would correspond to the pattern of infection of seedlings growing in soil. Spores of Gi. calospora and Gl. albidum were examined for germination in Millipore filter sandwiches after 7, 21 or 42 days and A. laevis and E. infrequens at 7 or 42 days in moistened untreated surface soil in the absence of plants. There were 25 spores of Gl. albidum, A. laevis and E. infrequens per sandwich and five spores of Gi. calospora with three replicates of each treatment.

Only spores of Gi. calospora germinated rapidly (Table 21). Spores of Gi. calospora may contribute most of the initial infection by that fungus. Clearly, spores of Gl. albidum, A. laevis and E. infrequens were not important in the initial stages of root infection. As initial infection of some seedlings occurred from about six days, it is likely that inoculum other than spores caused initial infection unless germination of spores is enhanced by the presence of plant roots. It is also likely that, except for Gi. calospora, spores are an unimportant source of initial infection of seedlings.

Table 21. Germination of spores of *Glomus albidum*, *Acaulospora laevis*, *Entrophospora infrequens* and *Gigaspora calospora* in Millipore filter sandwiches in moistened soil. (Mean of three replicates).

Fungus	Time to harvest		
	7 days	21 days	42 days
Glomus albidum	0/25	0/25	6/25
Acaulospora laevis	0/25	-	14/25
Entrophospora infrequens	0/25	-	6/25
Gigaspora calospora	4/5	3/5	4/5

Another way to examine the importance of spores as a source of initial infection from natural soil is to sieve dry soil and use each fraction as inoculum in tests of infectivity. If spores are the most important source of infection, then fractions that contain spores would be the main fractions to contribute to infection by that species. Sieve mesh sizes chosen were 50μm, 250μm and 1mm. Eight soil samples, each of 50g were sieved and the fractions collected. Four soil samples had been collected in autumn, 1984, and four in the preceding spring. Two samples at each collection were from 0 - 10cm and two from 30 - 40cm deep. Each fraction was placed 1 - 4 cm below the soil surface in the centre of pots of moistened autoclaved bulk soil amended with 5 μg/g P as Na_2HPO_4. P amendment of soil was used in an attempt to ensure maximum growth of roots through the soil with a consequent maximum interception by mycorrhizal fungi. In an earlier experiment, it appeared that P amendment of soil had a beneficial effect on root growth but no effect on the degree of mycorrhizal infection. One germinated seed each of Plantago drummondii and Podotheca angustifolia was transplanted into the inoculum in each pot. Plants were harvested after six weeks and the roots examined for mycorrhizas.

Spores of the coarse endophytes A. laevis, E. infrequens and G. albidum are between 50 and 250μm and Gi. calospora between 150 and 300μm diameter (Trappe, 1982) and may be found in all fractions greater than '>50μm'. In the surface soil CE occurred in all but the fine fraction (Table 22). However, A. laevis was found only in the '>50μm' fraction and E. infrequens was not observed. In the deeper soil, Gi. calospora was found in the coarse fraction, which was mostly root fragments. This indicates that except for A. laevis, fungal spores are not the only infective propagules at six weeks and that regrowth of hyphae from root fragments may have contributed to early infection observed elsewhere. As suggested by Tommerup and Abbott (1981), A. laevis may not survive in fragments of dry roots.

The presence of FE in each fraction (Table 22), though not each sample, suggested

Table 22. Vesicular-arbuscular mycorrhizal fungi present in the roots of Plantago drummondii and Podotheca angustifolia after six weeks in soil inoculated with soil sieved through a stack of sieves with mesh sizes of 1mm, 250µm, and 50µm.

Time of collect'n of soil sample	Rep.	Sample depth (cm)	Soil fraction >1mm		>250µm		>50µm		fine	
			CE	FE	CE	FE	CE	FE	CE	FE
Autumn, 1984	1	0-10	+	I	+	I	+	I	-	I
	2		-	I	-	I	-	I	-	I
Spring, 1983	1	0-10	+	I	+	I	-	I	-	I
	2		+	I	+	I	+	I	-	I
Autumn, 1984	1	30-40	-	-	+	II	-	II	-	-
	2		-	II	+	II	-	II	-	-
Spring, 1983	1	30-40	+	II	-	II	+	II	-	II
	2		-	-	-	-	-	II	-	II

+ = CE observed

I = FE I observed

II = FE II observed

- = no VAM observed.

that if spores of FE were present, they may not be the only infective unit of importance in establishing infections of FE, though spores of FE may have contaminated all fractions. Only single units of infection of FE II were found in plants in each pot.

Survival of Propagules of VAM Fungi Over Summer

Collections of soil from spring and autumn gave no clear indication of the effect of the stresses of summer on the survival of mycorrhizal fungi. A single harvest at six weeks gave little indication of the soil fractions of importance in the initiation of mycorrhizas and the absence of ectomycorrhizas on Podotheca angustifolia was unexpected, and may have been due to the amendment of the bulk soil with P. These factors were investigated together.

Samples of dry soil were collected in October, 1984 from 0 - 10cm and 30 - 40cm down the profile. Each soil sample was thoroughly mixed after breaking roots and crushing clods. Soil was treated in one of three ways. The control was left at room temperature in the laboratory, where temperatures fluctuated around 22^{o} C. The other treatments were placed in cloth sachets of about 150g of soil at either 2 - 3 cm or 30 cm below the soil surface in tubes 11 cm diameter by 30cm deep. The tubes were otherwise filled with field soil and placed vertically in the field with the top lip of the tube at the surface of the soil. Tubes were placed adjacent to the soil thermograph over the summer of 1984/5. The site was exposed to the sun for most of the day. There were five replicates of each treatment.

At harvest, the sachets of soil were recovered from the tubes and each treatment bulked. The size of the infective units was determined this time in soil that had not been amended with P. A sample of 50g of soil from each treatment was sieved and the fractions tested for infectivity at six, ten and 42 days after wetting the soil. P. angustifolia was the trap plant used. The effect of P on the infection by ectomycorrhizal fungi was examined in one further replicate of the soil treatment left in the laboratory. Phosphate (5µg/g) was mixed thoroughly through the soil and the pattern of mycorrhizas in the amended and unamended soils compared. Also, an MPN of the VAM

fungi, using P. drummondii, was harvested after 42 days.

No infection was found at six days. At 10 days CE, including Gi. calospora was found; in the untreated soil sample collected from 0 - 10cm deep, CE was found once in the 250µm fraction; in the untreated sample from 30 - 40cm deep in the 50µm and the fine fraction and the 250µm fraction placed at 30cm over summer. Gi. calospora was found in the untreated sample collected from 30 - 40cm deep in the 50µm fraction and the 1mm fraction placed at 30cm over summer. No CE or Gi. calospora was found in the soil kept at 2cm over summer. No FE was found. FM Asco I was the only ectomycorrhiza observed, on one plant in the nil treatment in the '>250µm' fraction. At 42 days (Table 23), several CE including Gi. calospora and both FE I and II had formed VAM.

The presence of CE in all fractions was unexpected. CE has not previously been observed to pass through a 50µm mesh sieve. The CE in the fine fraction (at 10 days only) was not identified. Spores of CE would, except some of Gi. calospora, be found in the 50 - 250µm fraction. As CE were observed in all fractions, it suggests that propagules other than spores were important for establishment of VAM. Infections by FE I and II occurred from different fractions. FE II was only found in the fine fraction, and this may have been due to there being few propagules present. FE I was found in coarser fractions. As it has been found in the fine fraction elsewhere (Table 22), it is possible that either there were few propagules present and/or that small propagules were less common than large propagules. If the fungus forms spores infrequently, and spores are the only part of the fungus to pass through a 50µm mesh, the later explanation is more likely.

Infections formed by Endogone or basidiomycetes were not observed. Of the ascomycetes observed, FM Asco I occurred in all but the coarse fraction. This suggests that regrowth of hyphae does not occur from dried roots and spores may be propagules of importance for the survival of this fungus. Insufficient infection by the other ascomycetes occurred to determine the relative importance of each fraction. For FM Asco II, though, infection occurred from the fraction that contained root pieces, suggesting that old ectomycorrhizas were a source of inoculum of the fungus.

The data from depth of collection of the inoculum suggests, once again, that Gi.

Table 23. Mycorrhizas on Podotheca angustifolia after 42 days in soil inoculated with sieved fractions of soil that had been collected from 0 - 4 or 30 - 40cm deep and placed at either 2, 30cm deep or kept in the laboratory over summer.

Source of inoculum	Depth of treatment	Myco-rrhizas	Soil fractions >1mm	>250µm	>50µm	fine
0 - 10 cm	nil	ecto	-	I	I	I
		CE	+	+	+	-
		FE	-	I	I	-
	2cm	ecto	-	-	-	I
		CE	+	+	+	-
		FE	-	I	I	-
	30cm	ecto	-	II	-	I
		CE	+	+	+	-
		FE	-	I	-	-
30 - 40cm	nil	ecto	-	-	-	-
		CE	+Gi	+Gi	-	-
		FE	-	-	-	II
	2cm	ecto	-	-	III	-
		CE	-	+	-	-
		FE	-	-	-	-
	30cm	ecto	II	I	-	I
		CE	+Gi	+Gi	-	-
		FE	-	-	-	-

ecto; I = FM Asco I, II = FM Asco II, III = FM Asco III.

CE; Gi = Gigaspora calospora present, + = other CE present

FE; I = FE I, II = FE II.

- = no mycorrhizal fungus observed.

calospora, FE I and II have restricted distributions in the soil; FE I in the surface soil and Gi. calospora and FE II lower in the soil profile. There seemed to be no stratification of FM Asco I and II, both being found in the surface and lower profiles.

Amendment of soil with phosphate and the placement of soil at the surface over summer markedly affected the fungi that formed mycorrhizas. In those soils amended with P, there were no ectomycorrhizas and Gi. calospora did not regrow from soil that had been placed at the surface during summer. Two factors may have caused the absence of Gi. calospora. The fungus may have been present at low concentrations in the soil such that by chance, no propagules were present in the soil placed at the surface or the fungus may have been killed by factors associated with the surface soil that are not found at depth.

The alternative explanations were examined in the MPN, where densities of Gi. calospora, other CE, FE I and II were estimated. As with the soil fractions, Gi. calospora was absent in soil that had been placed in the surface soil over summer (Table 24), though not in soil kept in the laboratory or that which was placed at 30cm over summer. FE II was also reduced by exposure to the effects of summer at the soil surface, though the reduction was not significant. Other groups of fungi were not significantly affected. It would seem, however, that there was sufficient inoculum of Gi. calospora present.

There are two factors associated directly with summer and at least one indirect factor that may explain the change in propagule density of Gi. calospora. High soil temperatures may kill propagules of Gi. calospora directly. Temperatures recorded at the soil surface reached 70^{o}C on at least one occasion and were above 65^{o}C for more than two hours on several occasions in 1984/5.

During summer, there were several brief falls of rain (Appendix 2). These may have been sufficient to germinate propagules of Gi. calospora. Unless there are fresh roots available for infection, and infection occurs, then hyphae produced from the propagules and maybe the propagules as well, may be killed by the rapid onset of soil drying that occurs during summer. This hypothesis is supported by evidence of Tommerup (pers. comm.) who observed that spores of Gi. calospora from pot culture need to be dried down very slowly to retain viability once water had been imbibed.

Table 24. MPN of propagules per 100g dry soil of Gigaspora calospora, other CE and FE in soil collected from 0 - 10 or 30 - 40cm deep and either placed at 2 or 30cm deep or kept in the laboratory over summer. (Mean with 95% confidence interval).

Source of inoculum	Depth of treatment	VAM fungi	MPN	95% confidence interval
0 - 10cm	nil	CE	26	11 - 60
		FE	5.5	2 - 13
	2cm	CE	34	15 - 79
		FE	7.8	3 - 18
	30cm	CE	12	5 - 29
		FE	3.9	1.4 - 9
30 - 40cm	nil	Gi. calospora	1.2	0.4 - 2.8
		CE	2.5	1.1 - 5.9
		FE	0.4	0.1 - 0.9
	2cm	Gi. calospora	0	
		CE	0.6	0.3 - 1.4
		FE	0.009	0.004 - 0.2
	30cm	Gi calospora	1.9	0.8 - 4.5
		CE	11.6	5 - 27
		FE	1.9	0.8 - 4.5

Changes in water potential at the soil surface are likely to be rapid in summer. Also, the presence of propagules of Gi. calospora below 20 cm in the soil profile would ensure germination of the propagules only when the profile was wet and roots more likely to be growing. Levels of available Mn in the soil may have been increased by the heat of summer and thus inhibited germination of propagules of Gi. calospora.

These factors were examined using a natural soil with relatively high levels of Gi. calospora (10 - 15 spores per 100g dry soil). The soil was treated in four different ways. Five hundred grammes of dry soil was either placed in an oven set at 70^{o}C for two hours; moistened to approximately -0.001MPa then air dried in a shallow tray in a room at about 20 - 25^{o}C; moistened, air dried and then heated as above; or left untreated. The MPN of Gi. calospora, other CE and FE were estimated in each soil, using P. drummondii as the trap plant. Roots were examined after 42 days growth.

The results (Table 25) show that Gi. calospora did not survive one wet/dry cycle. The vulnerability of Gi. calospora to wet/dry cycles may be due to the germination of all the propagules of the fungus when moistened. Other fungi have some spores that do not germinate within 42 days of wetting of soil (Table 21) and thus be likely to survive more than one wet/dry cycle. It is also possible that propagules that germinated, became dormant as the soil dried or that some hyphae in root pieces did not emerge when the soil was wet. Thus there was a reservoir of VAM fungi, apart from Gi. calospora, that survived the wet/dry cycle.

Dry heat by itself had no significant effect on the survival of any of the VAM fungi examined. After moistening and drying the soil, dry heat reduced numbers of propagules of FE and CE, though not significantly. The possibility of increased vulnerability to heat of propagules that had been moistened and dried was not pursued. It is possible that the survival of FE II was also reduced by the wet/dry cycles of summer. However, FE II was never sufficiently common to test directly, with any confidence, vulnerability of the fungus to wet/dry cycles.

Other means of surviving the stress of summer were not tested, though the presence of ungerminated spores of CE after 42 days in moist soil suggests that spores may be an important survival stage of those fungi.

Table 25. Most Probable Number (MPN) of propagules in soil of *Gigaspora calospora*, other coarse endophytes (CE) and fine endophytes (FE) treated with either heat, a wet/dry cycle, both or not treated using *Plantago drummondii* harvested after six weeks.

Treatment	Most Probable Number of propagules			
	FE	CE	*Gi. calospora*	Total
Control	0.12*	12	1.7	12
Heat; 70°C for two hours	0.16	7.1	1.3	7.1
Moistened then air dried	0.39	16	0	16
Moistened then air dried and then heated	0.003	5.4	0	5.4

*- 95% confidence interval is determined by dividing or multiplying the MPN value by a factor of 2.32 to obtain the lower and upper limits respectively.

The release of available Mn by summer heat was examined directly. A soil temperature of approximately 70°C was recorded several times just below the soil surface at the study site. Soil was collected within 24 hours of one such occasion (17.1.1985, Appendix II). Concentrations of extractable Mn (Table 16) were found to be less in the open, where soil temperatures reached nearly 70°C than under litter where the soil temperature reached 35°C. Presumably the inhibition of germination of propagules of VAM fungi by Mn is only of importance where there is also a significant reduction in the number of microbes. Increase in the availability of Mn due to heating of dry soil does not appear to be important in the field.

It is clear that two stresses associated with the seasonal aridity at the park, heat and occasional summer showers, have a negligible effect on the survival of most VAM fungi. The one species that is killed by rapid fluctuations of soil water over summer, Gi. calospora, is not found where these fluctuations occur. Other species of VAM fungi were not significantly affected by these stresses, possibly due to the presence of significant numbers of propagules which do not respond immediately to the presence of adequate soil water, or due to the abiltiy of germinated propagules to return to quiessence.

At the park spores seem to be of negligible importance in the initiation of infection of plants by VAM fungi. Few spores, including those of A. laevis which does not seem to have other forms of infective units, germinate within 42 days of the soil becoming moist. Indeed, even where there were relatively high numbers of spores of Gi. calospora (10 - 15 per 100g dry soil) that germinated rapidly (80% within 7 days, Table 21), an MPN of 1.7 propagules per 100g dry soil was observed (Table 25). This suggests that in soil at the study site, either spores were of minor importance in the initiation of VAM and/or that the MPN method underestimated the number of propagules. If the latter, then spores were of even less significance in the initiation of VAM in seedlings, than germination data indicate.

It is also likely that the infection of each species initiated by propagules other than spores would continue to occupy a predominant position in the root system due to prior occupancy (Smith & Walker, 1981; Wilson & Trinick, 1983 B; Abbott & Robson, 1984) of the roots. The predominance of occupancy by fungi from non-spore propagules

is one possible explanation for the few spores found in soil from the park. Fungi that infect the roots first are derived from non-spore sources. Early infection is of importance to the maintenance of predominance of infection. Thus species or isolates may be selected by factors other than spore formation. In agricultural soils, spores are advantaged by situations where delays in the appearance of roots after soil wetting (from the delay in sowing of crops after rainfall) necessitate either a larger nutrient reserve in the propagule or a delay in the onset of germination. However, as observed by Abbott and Robson (1984), competition between species of VAM fungi is determined by a wide range of factors and influenced by local conditions.

Much more work is required to determine if competition between fungi or soil factors control the distribution of spores. However, variations in the distribution of VAM fungi presumably reflect specific adaptations to the local environment.

Formation of Mycorrhizas

Previous work showed that the temperature of the soil influenced the rate of germination of seed of annuals at Ferries McDonald Conservation Park, with many seed germinating 10 - 15 days after sufficient rain in the autumn when temperatures were relatively low. Mycorrhizas have been observed on these plants but little is known about the initiation of mycorrhizas under different environmental conditions, including different temperatures.

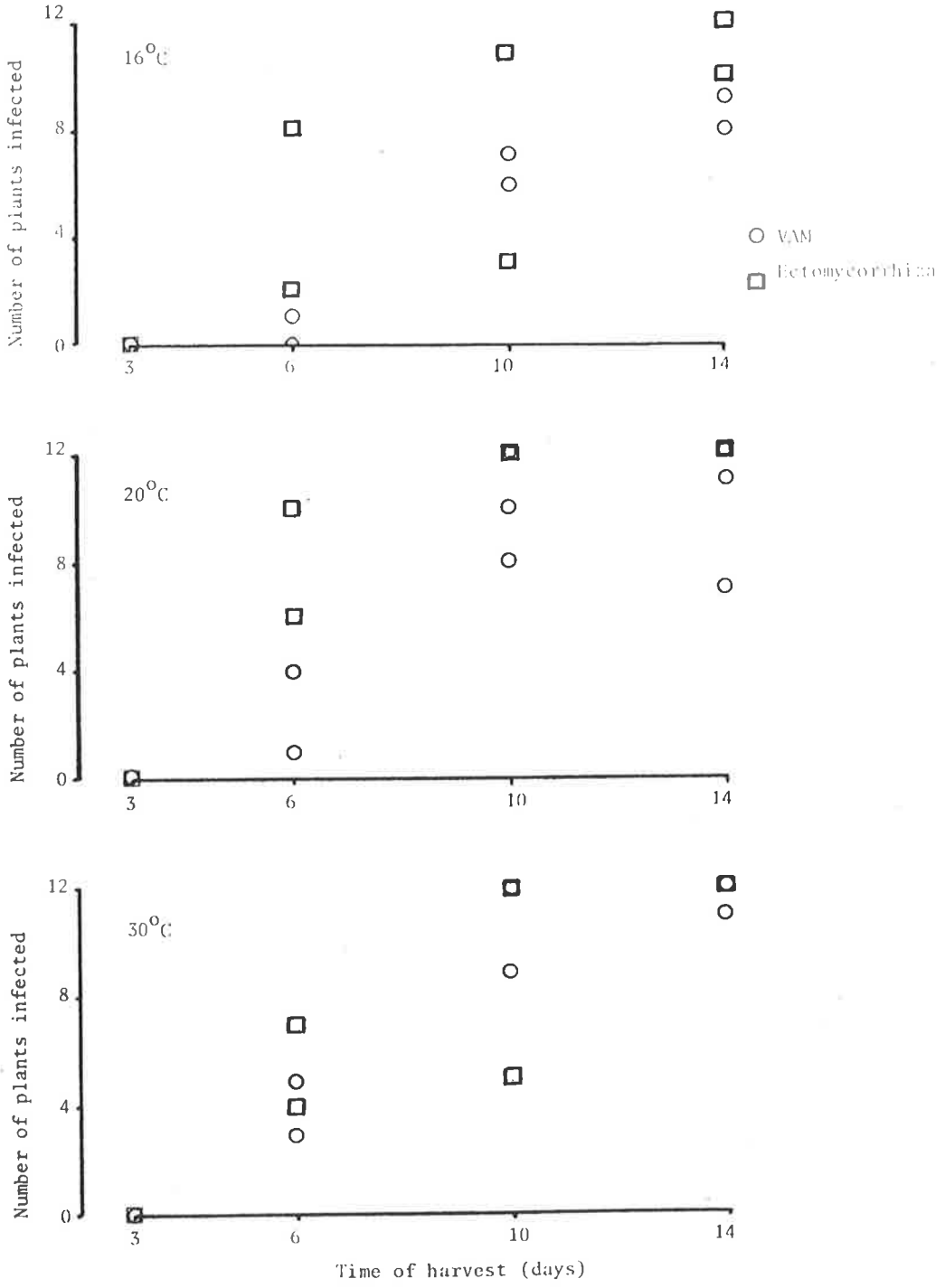
Effect of Temperature

The effect of temperature on the initiation of mycorrhizas was studied by growing seedlings in blocks of soil placed in Wisconsin tanks at different temperatures. Four seedlings of P. angustifolia with roots that were longer than 1.5cm were transplanted into each block of moistened soil. Pots were placed in Wisconsin tanks set at 16, 20 or 30^{o}C, three to be harvested after three, six, ten or 14 days. At harvest, the roots were examined for the presence of both VAM or ectomycorrhizas. The experiment was repeated and the results are presented together (Fig. 21).

No plants were mycorrhizal at three days. Of the ectomycorrhizal fungi, FM Asco I was observed at six days. FM Asco II and III were observed at ten days. FM Asco I was the only ectomycorrhizal fungus observed at 30^{o}C though all three ascomycetes were found at the lower temperatures. Ectomycorrhizas formed by Endogone sp. were seen at 16^{o}C and ectomycorrhizas formed by basidiomycetes were not observed.

Of the VAM fungi, mycorrhizas formed by Glomus albidum were observed at 16 and 20^{o}C at six days and FE at ten days. Acaulospora laevis and Entrophospora infrequens were not observed. The fungus that formed VAM at 30^{o}C after six days could not be identified. Infection by VAM fungi were less common at lower temperatures than by ectomycorrhizal fungi, but by 14 days, the difference was small. It is curious, however, that at 16^{o}C, there were fewer plants with VAM than at higher temperatures.

Fig.21. Vesicular-arbuscular and ectomycorrhizas on roots of 12 plants of Podotheca angustifolia after 3, 6, 10 or 14 days in blocks of soil at 16, 20 or 30°C. Data from two experiments.



It would appear that the optimum temperature for germination of seeds and germination and infection of roots from propagules of fungi differ.

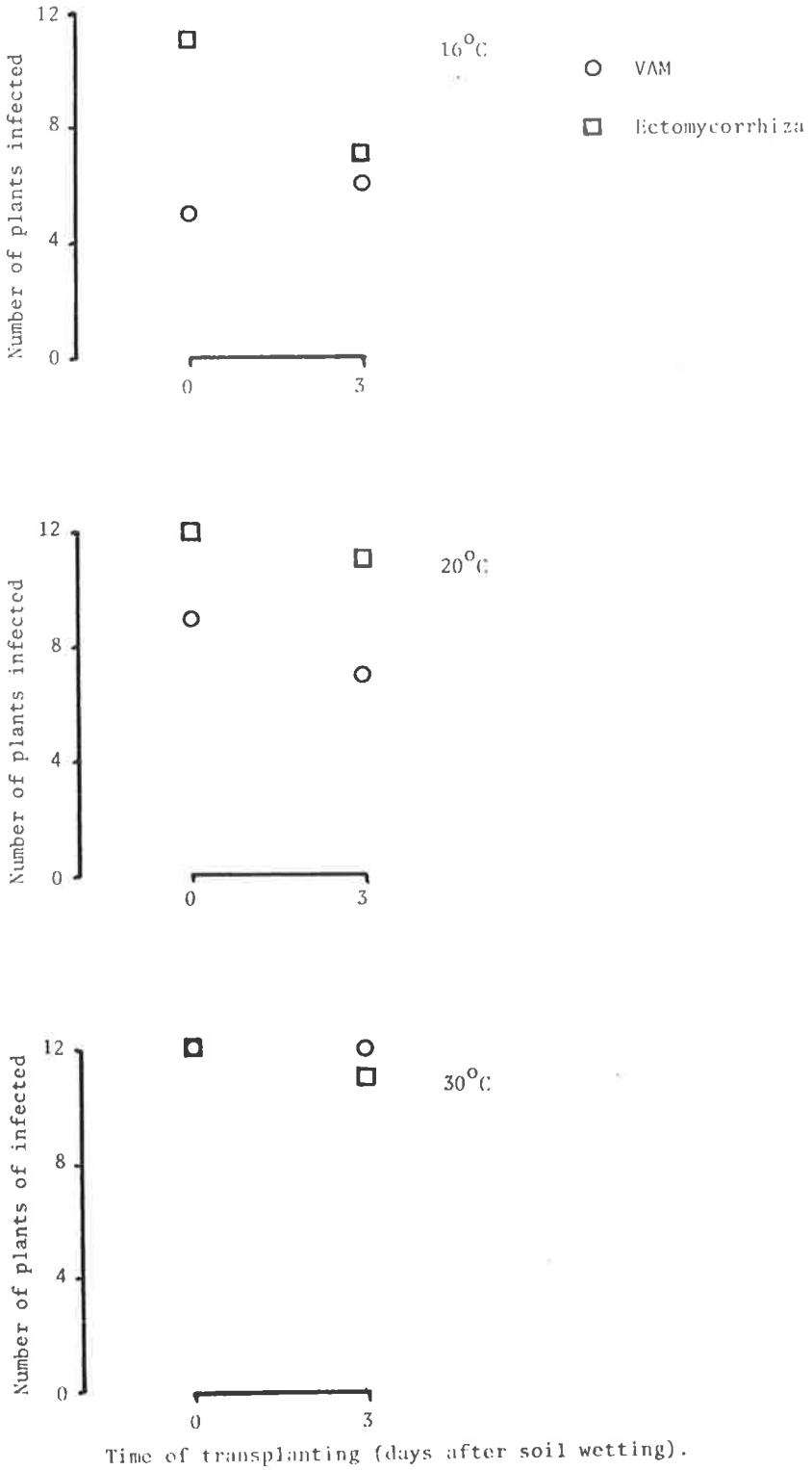
The rate of germination of propagules of mycorrhizal fungi may have been influenced by the use of a trap plant. To check this possibility, further blocks of soil were moistened. All seedlings of P. angustifolia were germinated at the same time. Six pots had three seedlings transplanted immediately and six pots after three days.Two pots from each date of transplantation were placed in waterbaths set at 16, 20 and 30^{o}C and all pots were harvested and examined for mycorrhizas ten days after wetting the soil. Results (Fig. 22) suggest that the presence of a host does not stimulate the germination of mycorrhizal fungi as infection of the host was similar after seven or ten days in soil that had been moistened at the same time.

At all temperatures, most seedlings were ectomycorrhizal by day 14. However, not all seedlings had VAM by day 14, particularly at 16^{o}C. Over the period it takes for seed to germinate, the soil moisture level can decrease markedly (Fig 16), changing from field capacity to permanent wilting point. Germination of seed in the field is slower than the germination of mycorrhizal fungi, particularly at 20^{o}C or higher. The possibility that propagules of mycorrhizal fungi will germinate in the absence of an appropriate host is high.

One major problem with the use of blocks of soil was the uneven distribution of soil moisture. When blocks were initially moistened, the water tended to run down the side of the pot and subsequently infiltrate the block from beneath. This was due to both the hydrophobic nature of the soil surface and the gap left at the edges of each block when the cutting cylinder was removed. To ensure a more even distribution of water through the soil, it was necessary to thoroughly mix water through the soil prior to potting. The distribution of roots through the blocks was also a problem. At harvest, roots of the seedlings became tangled with the now severed roots of perennials, making complete recovery of the test plant difficult. Thus it was necessary to either remove or break up all large fragments of root in the soil prior to transplanting seedlings.

Because of these problems, all subsequent experiments were done in a bulk soil collected from 0 - 15cm deep from the study site. The soil was thoroughly mixed and

Fig. 22. Vesicular-arbuscular and ectomycorrhizas on 12 plants of Podotheca angustifolia transplanted zero or three days after wetting the soil and grown for ten days in water baths at 16, 20 or 30°C.



passed through a 5mm mesh sieve. This may have altered the distribution of fungal propagules and the rate at which mycorrhizas were initiated.

To determine whether infection of plants in the mixed soil was substantially different from that in blocks, seedlings of P. angustifolia were grown for 10, 28 or 56 days in pots of moistened soil. Five replicate pots were placed in each water bath. At each harvest, the number of plants infected with CE, FE, FM Asco I and II and other ectomycorrhizal fungi were counted. At 28 and 56 days, the percent root length infected with VAM and ectomycorrhiza was estimated and the dry weight of shoots determined.

Though there were more plants with VAM and ectomycorrhizas (Table 26) in the mixed soil than in the blocks (Fig 21) after 10 days, this was not considered significant because the redistribution of fungal propagules more evenly through the mixed soil would have made it more likely that roots would have contacted infective propagules.

After ten days, there were similar numbers of plants infected with each type of mycorrhizal fungus in the mixed soil. Of the fungi observed, FM Asco I was again the only ectomycorrhizal fungus found at 30^{o}C. Spread of this fungus, though, was inhibited at this high temperature. A basidiomycete was observed on one plant at 56 days in the 16^{o}C treatment. Endogone sp and FM Asco III were not observed.

A. laevis and G. calospora were found at all temperatures, A. laevis from 28 days, Gi. calospora after 10 days. FE, E. infrequens and G. albidum were not observed. The most common VAM fungus at 30^{o}C was not identified, though appeared to be the same fungus that occurred in the blocks of soil.

Plant growth differed at different temperatures. Seedlings at 20^{o}C were significantly heavier at 28 days, than at other temperatures, though the difference was not significant. By 56 days, plants in the 20^{o}C water bath were significantly heavier, so further experiments were harvested at 28 days to reduce the effect of different sized plants on the results.

Table 26. Vesicular-arbuscular and ectomycorrhizas observed on Podotheca angustifolia after 10, 28 or 56 days in mixed soil at 16, 20 or 30°C and mean shoot dry weight and percent root length infected with VAM and ectomycorrhizas after 28 or 56 days.

Temp °C	VAM CE /5 plants	Ectomycorrhiza Asc I	Asc II	Total	Shoot dry weight (x ± SEM) g x 10^{-2}	Percent root length VAM	Ect
					Day 10		
16	5	4	2	5			
20	5	5	4	5			
30	5	4	0	4			
					Day 28		
16	5	5	2	5	0.5 ± 0.06	40	40
20	5	5	3	5	0.6 ± 0.13	54	36
30	5	5	0	5	0.4 ± 0.03	45	2
					Day 56		
16	5	5	4	5	2.4 ± 0.23	80	30
20	5	5	4	5	4.7 ± 0.59	46	70
30	5	5	0	5	2.4 ± 0.23	68	12

It was apparent that different groups of fungi initiated mycorrhizal infection of the root under different conditions. Prior occupation of the root by one mycorrhizal fungus, may lead to the reduced presence of another. Thus the possibility that VAM fungi competed with ectomycorrhizal fungi for initial infection of roots of P. angustifolia was investigated. After roots become mycorrhizal, other types of competition may occur (Wilson, 1984), but secondary competition was not examined here.

Competition for initial infection of roots between VAM and ectomycorrhizal fungi was tested by estimating the number of propagules of VAM fungi that infect a host that forms only VAM and a host that forms both VAM and ectomycorrhiza in a soil that had propagules of both types of fungi. An MPN estimate of the VAM fungi in Pl. drummondii and the VAM fungi and ectomycorrhizal fungi in P. angustifolia were compared. Plants were grown for 28 days. Roots were examined for CE, FE, FM Asco I, II, III and basidiomycetes at harvest.

The result (Table 27) showed that there was no diminution of VAM on P. angustifolia in the presence of ectomycorrhizas compared with Pl. drummondii. There were also more propagules of mycorrhizal fungi available for infection of P. angustifolia than of Pl. drummondii, though whether this was of any advantage to the dually infected plant was unclear. There was no comparable test to determine if ectomycorrhiza were inhibited by the presence of VAM. No ectomycorrhizal host was found that became infected within 10 days of germination and that did not also become VAM. Species tested included Acacia longifolia (Andrews) Willd.,A. paradoxa D.C., Betula sp., Eucalyptus fasciculosa, E. incrassata, E. rugosa, Melaleuca acuminata F. Muell., M. uncinata and Quercus sp..

Table 27. Most Probable Number of propagules per 100g dry soil, with 95% confidence interval, of vesicular-arbuscular mycorrhizal and ectomycorrhizal fungi found using Podotheca angustifolia and vesicular-arbuscular mycorrhizal fungi using Plantago drummondii harvested at 28days.

Host	Mycorrhizal fungi	MPN	Confidence interval
Plantago drummondii	CE	13.3	5.7 - 30.9
	Total VAM	13.3	5.7 - 30.9
Podotheca angustifolia	CE	19.7	8.5 - 45.7
	Total VAM	19.7	8.5 - 45.7
	FM Asco I	17.0	7.3 - 39.4
	FM Asco II	0.3	0.1 - 0.7
	FM Asco III	0.2	0.1 - 0.6
	All	27.5	11.8 - 63.8

Soil water content fluctuated markedly during the initial growth phases of a season (Fig. 16). These fluctuations may have inhibited germination of mycorrhizal fungi. In a pilot experiment to examine the effect of soil water content on the initiation of mycorrhizas, infection of seedlings of both P. angustifolia and M. uncinata in pots of soil moistened to either 6, 8, 10 or 12% was examined. There were four replicate plants harvested after either 14 or 28 days in the growth room. Soil water was maintained by placing a lid of a petri dish over the pot, a proceedure that may have reduced photon irradiance. Soil water potential was checked at the start and the end of the experiment using the filter paper method.

Mycorrhizas found on the roots at harvest showed a change in the proportion of each type of mycorrhiza on both hosts with change in soil water content (Table 28), and though the initiation and development of mycorrhizas was similar in the two hosts, the variation in development on P. angustifolia was more extreme, probably due to the considerably longer roots of this species. The soil water potential at 6% soil moisture was - 0.006, at 8% was - 0.004 and at 10 and 12% was -.0.001MPa. As the soil water content at - 1.5MPa was between 3 and 4.5% for soil samples collected from the surface and a soil water content of 12% has not been found in any of the samples returned to the laboratory, the next experiment used soil water contents of 5, 7.5 and 10%.

Seedlings of P. angustifolia were transplanted into pots of moistened soil and placed into Wisconsin tanks set at 16, 20 or 30^{o}C. Six replicates of each treatment were harvested at 28 days. At harvest, the presence of VAM and ectomycorrhizas was checked on each root system, percent VAM and ectomycorrhizas estimated and the dry weight of shoots determined. Soil water potential of each pot was measured at the beginning and the end of the experiment. Again, soil water content was retained by placing lids of plastic petri dishes over each pot. Soil water potential at a soil water content of 5% was approximately - 0.008, at 7.5% was - 0.006 and at 10% was - 0.001MPa.

Contrary to the previous experiment, soil water potential had no effect on the

Table 28. Vesicular-arbuscular mycorrhizas and ectomycorrhizas found on Podotheca angustifolia and Melaleuca uncinata grown in mixed soil at 12, 10, 8 or 6% water content for 14 or 28 days in the growth room.

Host plant	Water content	VAM /4 plants	Ecto	%VAM	%Ecto
		14 days			
P. angustifolia	6%	4	4		
	8	3	4		
	10	4	4		
	12	4	4		
M. uncinata	6	1	2		
	8	2	4		
	10	4	4		
	12	4	2		
		28 days			
P. angustifolia	6	4	4	10*	80
	8	4	4	23	74
	10	4	4	48	50
	12	4	3	73	23
M. uncinata	6	3	4	23	48
	8	4	4	40	38
	10	4	4	58	23
	12	4	4	50	25

* - mean of four replicates.

initiation and development of mycorrhizal infection (Table 29) at any temperature. High soil temperatures again reduced spread of infection of FM Asco I, the only ectomycorrhizal fungus found at 30°C. At 30°C there was also a slight increase in the percent root length infected with VAM and a significantly lower growth of shoots compared to plants grown at lower temperatures.

These data (Table 29) differ from previous experiments where the effect of soil water content (Table 28) and soil temperature (Table 26) were examined. Where soil water content was varied for plants grown in the growth room (Table 28), at 28 days on P. angustifolium there was a marked coincidence of type of mycorrhiza with increase in soil water content. Differences in photon irradiance may have influenced the result (Tester et al, 1986) but it is difficult to explain how. Also, where soil temperature was varied (Table 26) at 28 days there was a lack of coincidence of results that occurred later (Table 29). The result of these three experiments suggest that variation in initiation of infection by mycorrhizal fungi is considerable, and that there may be significant competition between VAM and ectomycorrhizal fungi to establish mycorrhizas.

Table 29. Dry weight of shoots of and vesicular-arbuscular and ectomycorrhizas on Podotheca angustifolia grown in mixed soil with a soil water content of either 5, 7.5 or 10% in pots in water baths set at 16, 20 or 30°C for 28 days. (Mean of 6 plants).

Temp.	Soil water content	VAM	Ect	%VAM	%Ect	Shoot dry weight mg ($\bar{x}$ ± SEM)
16°C	5%	6/6	6/6	22	70	4.7 ± 0.8
	7.5	6	6	23	68	5.4 ± 1.0
	10	6	6	25	73	4.6 ± 0.6
20	5	6	6	35	57	5.4 ± 0.6
	7.5	6	6	27	63	5.4 ± 0.8
	10	6	6	38	57	4.4 ± 1.0
30	5	5/5*	5	52	6	3.5 ± 0.2
	7.5	6	6	65	2	3.3 ± 0.2
	10	6	6	65	5	3.9 ± 0.4

*one plant died prior to harvest.

VAM and ectomycorrhizas were usually both present on the root system of P. angustifolia from the field and the laboratory. In some instances, VAM would underlie ectomycorrhizas, with the appressoria of the VAM fungus surrounded by the ectomycorrhizal sheath on a segment of root. These observations suggest that there may be, under some circumstances, a synergistic growth response in the presence of both types of mycorrhiza. They also suggest that there may be competition between VAM and ectomycorrhizal fungi during colonization of the root, as occurs between species of VAM fungi.

Before the growth of doubly infected mycorrhizal plants could be examined, it was necessary to determine which fungal species to use and how much inoculum was required. The only identified species of ascomycete was Peziza whitei. As it was in pot culture and it gave a reasonable growth response in pot culture, no other ectomycorrhizal fungus was considered.

The plant growth response to infection by the VAM fungi A. laevis, Gi. calospora, Gl. albidum and FE was compared. Seedlings of P. angustifolia were inoculated with 0.5g mycorrhizal root in autoclaved bulk soil. There were six replicates harvested after six weeks in the growth room. At harvest, plants inoculated with A. laevis had the highest growth response (Table 30), though the growth responses of the five treatments were not significantly different.

To determine the amount of inoculum required for each mycorrhizal fungus, seedlings of P. angustifolia were inoculated with either 0, 0.1, 0.25, 0.5 or 1g of roots of M. uncinata infected with P. whitei or 0, 0.1, 0.25, 0.5 or 1g of roots of T. subterraneum infected with A. laevis. Four plants of each treatment were harvested at either three, six or ten days and the roots examined for the presence or absence of mycorrhizas. From the results (Table 31), an equivalent rate of infection arose from several combinations, the least inoculum being 0.5g of roots infected with A. laevis and 0.25g of P. whitei.

The growth response of P. angustifolia to dual inoculation was examined. There

Table 30. Dry weight of shoots of *Podotheca angustifolia* inoculated with *Acaulospora laevis*, *Gigaspora calospora*, *Glomus albidum*, FE or uninoculated after six weeks in the growth room. (Mean * SEM of 6 reps)

Fungus	Shoot dry weight ($g \times 10^{-4}$)
Nil	125 * 32
A. laevis	169 * 44
Gi. calospora	156 * 29
G. albidum	120 * 14
FE	135 * 18

Table 31. Presence of mycorrhizas on *Podotheca angustifolia* at three, six or ten days after inoculation with 0, 0.1, 0.25, 0.5 or 1g of roots of *Melaleuca uncinata* infected with *Peziza whitei* or of roots of *Trifolium subterraneum* infected with *Acaulospora laevis*. (four plants).

Quantity of root inoculum.	Fungus	Number of seedlings infected (out of four) harvested at		
		3 days	6	10
0g		0	0	0
0.1	*A. leavis*	0	1	4
	P. whitei	0	3	4
0.25	*A. laevis*	0	1	4
	P. whitei	0	4	4
0.5	*A. laevis*	0	4	4
	P. whitei	1	4	3
1	*A. laevis*	0	4	4
	P. whitei	0	4	4

were four treatments, inoculation with A. laevis, inoculation with P. whitei, inoculation with A. laevis and P. whitei and a control of uninfected mixed roots. There were ten replicates of each treatment. Plants were grown for six weeks in the growth room. At harvest root length, shoot dry weight, length of VAM and length of ectomycorrhizas were determined and the percent root length infected with VAM and ectomycorrhizas, calculated. At harvest, eight plants singly inoculated and seven plants doubly inoculated were found to be infected by A. laevis, and nine plants singly inoculated and ten plants doubly inoculated were found to be ectomycorrhizal.

There were four trends apparent in the parameters determined (Table 32). A. laevis did not significantly increase shoot dry weight and root length, either alone or in association with P. whitei. P. whitei, on the other hand, significantly increased both shoot dry weight and root length of the host ($p > 0.05$) both alone and in association with A. laevis. The percentage root length infected with A. laevis significantly declined ($P < 0.025$, Mann Whitney non-parametric test) in the presence of P. whitei, though when measured as total root length infected , the decline was significant only at $p < 0.1$. The percentage root length infected with P. whitei was not significantly affected by the presence of A. laevis on those plants that became infected with A. laevis.

In these results there was, again, a high degree of variability, for instance with the length of infection by A. laevis alone, the standard deviation of 120 was greater than the mean of 88cm, due mostly to two extreme observations of 380 and 8 cm, zero not included. Under the circumstances it is surprising that significant results occurred. The data can be interpreted, however, as indicating that there is competition between VA and ectomycorrhizal fungi for the spread of infection of the root. The data do not indicate whether this competition acts prior to infection or during the development of the infection units, though the MPN data (Table 27) suggest the later mechanism.

There was an insignificant increase in shoot dry weight after infection with both VAM and ectomycorrhizal fungi compared to infection with P. whitei alone. Unless a VAM fungus is found at the park that gives significant growth responses within about six weeks of inoculation to infection in pot culture in the growth room, the possibility of a synergistic growth response cannot be tested.

Table 32. Dry weight of shoots, length of roots, length of VAM and ectomycorrhizas and percent root length infected with VAM and ectomycorrhizas of *Podotheca angustifolia* inoculated with *Acaulospora laevis*, *Peziza whitei*, both or neither after six weeks.

Inoculum	Mean	SEM	Number
	Dry weight of shoots ($g \times 10^{-4}$)		
Nil	144	18	10
A. laevis	158	26	10
P. whitei	490	38	10
A. laevis + *P. whitei*	496	35	10
	Length of roots (cm)		
Nil	402	43	10
A. laevis	428	68	10
P. whitei	1736	274	10
A. laevis + *P. whitei*	1461	177	10
	Length of VAM (cm)		
A. laevis	88	42	8
A. laevis + *P. whitei*	29	9	7
	Length of ectomycorrhizas (cm)		
P. whitei	292	63	9
A. laevis + *P. whitei*	198	30	10
	% Root length infected with VAM		
A. laevis	16.3	4.3	8
A. laevis + *P. whitei*	2.3	0.7	7
	% Root length infected with ectomycorrhizas		
P. whitei	14.9	2.3	9
A. laevis + *P. whitei*	13.1	1.0	10

Mycorrhizal Associations of Thysanotus

The fungal associations of the roots of Thysanotus (Chapter 4) did not fit readily into any group. This may have been because flowering of Thysanotus occurs in spring/summer and roots collected were atypical or because Thysanotus has an undescribed mycorrhizal association. To determine if the association was mycorrhizal and gave growth response of the host, naturally occurring and synthsised associations were investigated.

The Plants

Two species of the monocotyledon Thysanotus occur at the park. T. patersonii is a climber that grows annually from a perennating bud on a cluster of tubers buried several cm below the surface of the soil. The twining shoot flowers annually and dies back in unfavourable conditions. Tubers are formed below ground on adventitious roots and are completely replaced annually during vegetative growth, with usually an annual increase in the number of tubers (Brittan, 1981; Pate & Dixon, 1981). T. juncifolious is a rush-like plant without leaves when mature. Stems grow annually from a perennial rhizome (Brittan, 1981). Two further species were used in these investigations. T. multiflorus, a plant with perennial grass-like leaves growing from a rhizome just below ground level. It is found naturally in the south-western part of Western Australia. Seed was obtained from commercial sources. T. tenellus, a plant with annual grass-like leaves growing from a cluster of tubers is found on the Eyre Peninsula of South Australia. Seed was obtained from a plant collected from near Port Lincoln and grown on in the glass house.

Roots of Thysanotus were collected from the park for observations during flowering, September/October for T. patersonii and November/January for T. juncifolius. Further collections of roots of T. patersonii were made from Belair, Blewitt Springs and Stirling, of T. juncifolia from Blewitt Springs and a collection of T. tenellus from near Port Lincoln.

Morphology of the Mycorrhizas of Thysanotus

Roots of plants collected from the field were cleared, stained in Trypan Blue and examined for mycorrhizas. Initial collections were of mature roots of T. patersonii and T. juncifolius from the park. The morphology of infection was difficult to observe as the epidermis had always separated from the cortex leaving a sheath of hyphae on the surface of the cortex (Fig 23). While most of the hyphae appeared to be septate, aseptate hyphae were also present. There appeared to be fungal structures in the cortex but these were difficult to observe in root squashes.

Seedlings of T. patersonii were grown in pot cultures of M. uncinata infected with either WARH 24 (an ascomycete from Western Australia; Warcup, 1985), Glomus fasciculatum or Glomus microcarpum Tul. & Tul. for 12 weeks. The morphology of all infections was difficult to observe in the root squashes. However, infection by the VAM fungi was extensive except for a zone about 7 - 9mm behind the root tip which was apparently free of infection. Just beyond this zone, there were what appeared to be distorted arbuscules, presumably in the cortex. There were no further arbuscules observed in the infected part of the root. Only a few vesicles were present, these always near the appressoria. Appressoria on the root tip were aborted. Penetration of the root was always between the epidermal cells, from hyphae which grew along the root in the junctions of the epidermal cells. Occasionally there were fan shaped structures between epidermal cells. The outer cortex was comprised of rows of long cells separated by short cells in between rows of long cells (see Fig. 25). Parallel hyphae with few cross branches usually covered the outer surface of long cells of the cortex. Short cells remained free of hyphae.

With WARH 24, occasional epidermal cells were covered by a sheath of hyphae, but no patches were observed that covered more than one cell. Hyphae penetrated between epidermal cells and formed partial sheaths between epidermis and cortex similar to those formed by VAM fungi. Short cells of the cortex were, as with VAM fungi, free of hyphae. In several pots, WARH 24 formed a sclerotium-like structure between

the epidermis and cortex, which broke through the epidermis. The structure comprised barrel-shaped cells filled with oil drops. Walls of the hyphae in the sclerotia were thin and hyaline. Sclerotia have not been observed in single pot cultures of WARH 24. WARH 24 can form thick walled hyphae in soil of single pot cultures with an ectomycorrhizal host, but these were not observed in the dual pot cultures. This suggests that the physiology of the association between Thysanotus and WARH 24 differs from typical ectomycorrhizas.

When grown in the presence of mycorrhizal

T. tenellus, T. juncifolius and T. multiflorus formed similar associations ~~in the presence~~ of a companion plant. However, only those roots that were growing in or near mycorrhizal roots of the companion plant were found with fungal infections. Away from mycorrhizas of the companion plant, roots of these species of Thysanotus were not mycorrhizal, as far as could be determined.

Embedding, sectioning and examination of roots and mycorrhizas of Thysanotus

The roots of Thysanotus were flexible and most tore when attempts were made to section them by hand. Those sections which were cut successfully were too thick for satisfactory examination. In order to examine in detail the morphology of the mycorrhizal associations of Thysanotus, it was necessary to embed specimens and cut sections with a microtome. On the advice of Dr A.E. Ashford, Department of Botany, University of New South Wales, the following proceedure was used.

Roots were washed from moist soil and placed immediately in deionised water. Segments of root about 0.2 to 0.4cm long were cut from selected roots and placed in 3% gluteraldehyde in 0.2M potassium phosphate buffer at pH 6.8. Fixation was overnight at $4^{o}C$. Segments were then rinsed and stored in phosphate buffer. Some roots were postfixed in 1% OsO_4 in phosphate buffer for two hours. Root segments were dehydrated in an ethanol series from 5% to 100% in ten steps, two hours per step, with overnight storage in 50% ethanol. Infiltration with resin was in three steps, two hours per step, with roots in London Resin (L.R.) White for 12 to 14 days at $4^{o}C$, while being agitated.

Polymerisation was under nitrogen gas and U.V. light at room temperature for 10 to 12 hours. Sections were cut with a glass knife, stained with Toluidine Blue at pH4.4 or Amide black and examined microscopically.

Young roots of T. patersonii collected from the park and roots of mycorrhizal T. tenellus, T. juncifolius, T. multiflorus and T. patersonii from pot cultures with G. clarum or P. whitei (P. 122 for methods) and non-mycorrhizal T. patersonii from pot culture in sterile sand were used.

In uninfected roots of Thysanotus (Fig. 24) there was a single layer of irregular epidermal cells surrounding the cortex. Large air spaces between the two tissues were common. In the outer cortex there were long and short cells. The short cells were always in close contact with the epidermal layer (Figure 25). Staining reaction of the two tissues differed. Walls of the epidermal cells stained with Toluidine Blue were blue in colour suggesting lignification of the cell walls. Walls of the cortical cells were purple suggesting an absence of phenolic substances. Sections stained with Amide Black showed heavy deposits between the cells of the outer cortex, ~~suggesting that nutrients moved into rather than between the cortical cells.~~

In mycorrhizal roots (Figs. 26,27), hyphae penetrated between epidermal cells and proliferated within the air spaces. There was a decrease in the diameter of hyphae. In only one case was penetration of the cortex observed. A single hypha of G. clarum was found in one cell in this instance. No arbuscules were observed. Hyphae penetrated into the zone of elongation of the root tip between the cortex and the epidermis (Fig. 25). Staining reaction of mycorrhizal roots was similar to uninfected roots. Hyphae of both G. clarum P. whitei ~~VAM~~ and ~~ectomycorrhizal~~ fungi stained blue with Toluidine Blue and remained unstained with Amide Black. Use of Toluidine Blue also showed a blue deposit surrounding the hyphae of all fungi (Fig. 28) between the tissues suggesting exudation of phenolic substances by the fungi.

Fig. 23. Surface of a root of Thysanotus patersonii collected from Ferries McDonald Conservation Park at flowering. The epidermis is missing and the 'sheath' of mycorrhizal fungi is peeling from the cortex of the root. 1cm = 80um

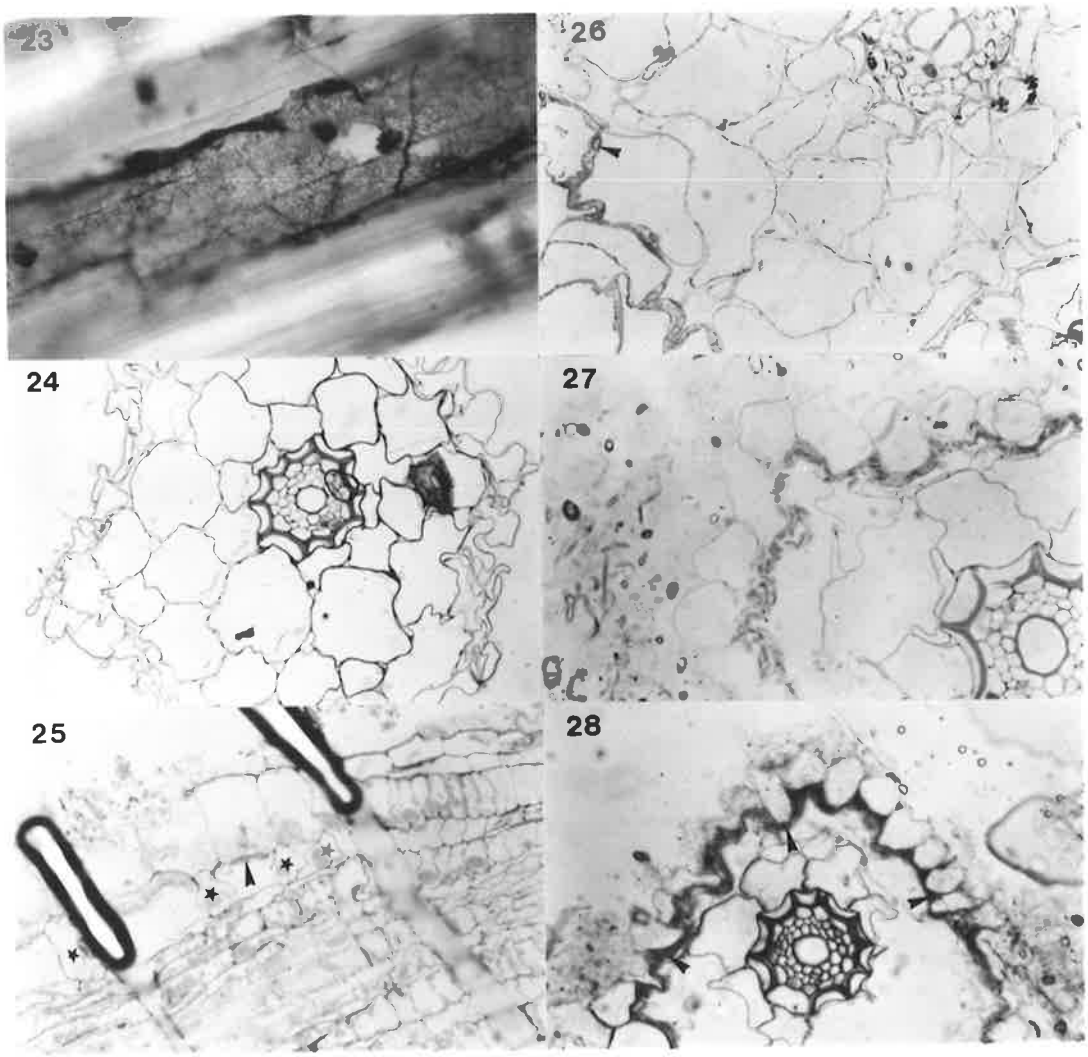
Fig. 24. T.S. of a non-mycorrhizal root of Thysanotus tenellus. 1cm = 80um

Fig. 25. L.S. of the tip of a root of Thysanotus juncifolius infected with Glomus clarum. Short cells (star) of the cortex are attached to the epidermis. Infection commences between the epidermis and cortex (arrowed) in the zone of elongation of the root. 1cm = 80um

Fig. 26. T.S. of a young root of Thysanotus patersonii collected from the park. Mycorrhizal fungi (arrowed) are between the epidermis and cortex. 1cm = 45um

Fig. 27. T.S. of a root of Thysanotus tenellus grown in pot culture with Glomus clarum. 1cm = 45um

Fig. 28. Blue stained exudate (arrowed) between the hyphae of Glomus clarum in Thysanotus juncifolius. 1cm = 80um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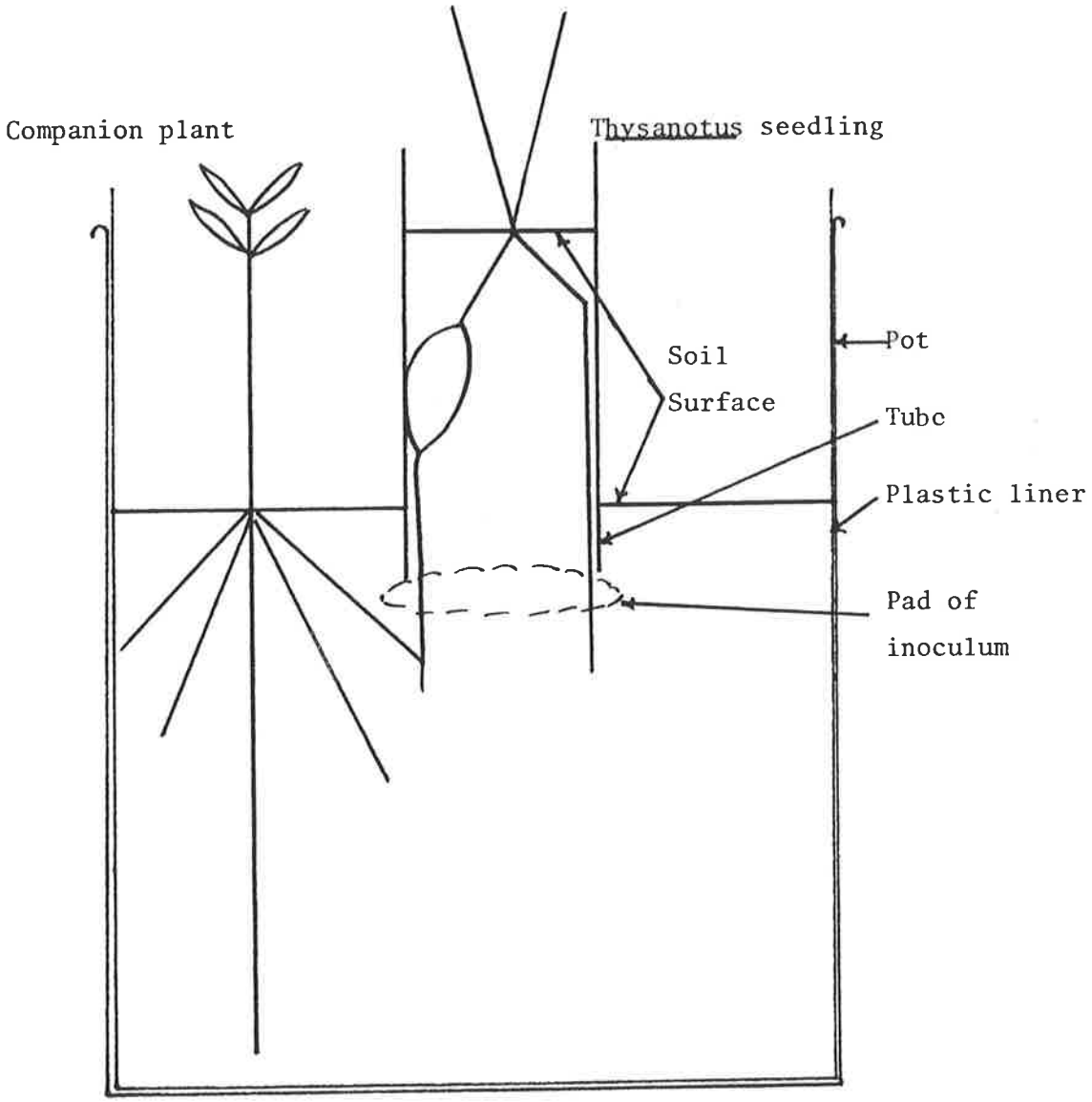
Tests to determine if there was growth response of Thysanotus to mycorrhizal infection were commenced prior to a suitable experimental soil from the park being found. Thus a mixture of 90% autoclaved coarse washed river sand and 10% autoclaved forest soil (Warcup & McGee, 1983) was used for all experiments with mycorrhizal Thysanotus. Seedlings of Thysanotus patersonii were transplanted into pots of soil over 0.5g chopped roots of M. uncinata infected with either WARH 24, Amanita sp (FM 2) or Glomus microcarpum or were transplanted without inoculum. There were six replicates of each treatment. After 12 weeks, all plants that survived were uninfected. The experiment was repeated using Laccaria ohiensis instead of FM 2, and again those seedlings that survived to harvest were uninfected.

It was noted that seed of T. patersonii germinated to form a single contractile root that pulled the shoot into the soil. The crown thus formed from below the soil surface. Later adventitious roots formed from the crown, but as the crown was 2 - 4cm below the soil surface,the new roots were below the inoculum in the inoculation experiments. Other roots from the crown grew laterally, initially, and secondary roots often commenced a further two to four cm below the crown. In most instances secondary roots formed close to the bottom of the pot. In contrast, seedlings of Thysanotus placed in pot culture with mycorrhizal companion plants, all became mycorrhizal.

Seedlings of T. tenellus also have contractile roots but the crowns of the plants were not found so deep in the soil. Seedlings of T. tenellus were transplanted to pots without inoculum, to tubes (Fig. 29) in pots over 0.5g of mycorrhizal root of M. uncinata, or in tubes adjacent to a mycorrhizal seedling of M. uncinata. Fungi used were Peziza whitei and Glomus clarum Nicol. & Schenck. There were six replicates of each treatment. Plants were harvested after eight weeks. Roots were examined for mycorrhizas and degree of infection determined using the following scale:
zero, no infection; one, infection only at the pad of inoculum; two, infection beyond the inoculum, but not to the bottom of the pot; three, infection extensive and to the bottom of the pot. Presence of tubers was noted and the number of leaves longer than one cm plus the total length of the leaves determined. Shoots and roots were then oven dried and



Drawing to scale

Fig. 29. Diagram of the system used to inoculate and grow mycorrhizal seedlings of Thysanotus.

weighed.

By harvest, two plants from the control and one inoculated with G. clarum had died. One further plant inoculated with G. clarum was uninfected. Mycorrhizas were formed where the inoculum was placed below the ultimate crown of the plant. Growth of T. tenellus (Table 33) was variable. Root and total weight of mycorrhizal plants were consistantly greater than non-mycorrhizal ones. Differences occurred in the growth of shoots. The number of leaves showed clear differences between mycorrhizal and non-mycorrhizal plants, though mixed results were obtained for total leaf length and shoot dry weight.

There was better infection of roots of T. tenellus in the presence of a companion plant. Only in the presence of a companion plant did infection of roots spread to the bottom of the pot. The difference in spread of mycorrhizas between G. clarum and P. whitei may reflect differences in the growth of hyphae in soil and on roots, with P. whitei more likely to grow along the surface of a root that G. clarum. Even with better spread of mycorrhizas in the presence of a companion plant, growth of mycorrhizal T. tenellus in the presence of a companion plant was not necessarily greater. T. tenellus infected with G. clarum was heavier in the presence than in the absence of a companion plant, but not when infected with P. whitei, where growth was similar. There are two possible explanations for variation in shoot growth.

In plants grown with an ectomycorrhizal companion, there was a possible lack of nutrients which restricted growth of Thysanotus. The ectomycorrhizal companion plants were twice as high as the VAM companion plants at harvest. As well, in this experiment there were no tubers formed with the ectomycorrhizal companion. In subsequent experiments, the size of the companion plant was observed to be critical to the development of Thysanotus. Where the companion had ceased growth prior to transplanting a seedling of Thysanotus, the seedling would only develop three short leaves. On the other hand, seedlings transplanted with very small companion plants did not become mycorrhizal for some time due to a lack of contact between the roots of Thysanotus and mycorrhizas of the companion plant.

Differences in shoot growth may also have been caused by the degree of spread of

Table 33. Growth and mycorrhizal infection of Thysanotus tenellus either inoculated or uninoculated by chopped roots or with a companion plant (Melaleuca uncinata) infected with Peziza whitei or Glomus clarum. Mean * SEM.

Treatment	Nil[+]	Inoculated			
		Roots		M. uncinata	
		P.w.	G.c.*	P.w.	G.c.
Mean Degree of infection[#]	0	1.8	0.8	2.2	2.5
Mean Number of tubers	0	0	0	0	0.3
	1.5	2.6	2	2	3
Total leaf lngth cm	11	21	10	12.6	24.3
Root dry wt $gx10^{-3}$	3*0.8	10*1.4	6*0.5	8*0.9	11*3.9
Shoot dry wt $gx10^{-3}$	4*0.6	6*0.9	4*0.3	4*0.2	8*1.9
Total dry wt $gx10^{-3}$	7*1.2	16*2.3	10*0.6	12*1.1	19*5.9
Root/shoot ratio	0.79	1.54	1.81	2.22	1.44

[+] = mean of four replicates

* = mean of four replicates, uninfected plants excluded; other infected plants, mean of six replicates.

[#] = 0 = no infection; 1 = infection limited to the pad of inoculum; 2 = infection beyond the pad of inoculum, but not to the bottom of the pot; 3 = infection to the bottom of the pot.

VAM fungi, with minimum infection of Thysanotus resulting in poor shoot growth compared with extensive development of infection as occurred in the presence of a companion plant.

Even with the disturbance to shoot growth of some Thysanotus, the root/shoot ratio of T. tenellus grown with a VAM companion or over a pad of ectomycorrhizal inoculum, showed a marked increase in the proportion of the plant found as roots. This is contrary to the commonly observed decrease in the root/shoot ratio of mycorrhizal over non-mycorrhizal plants (Smith, 1980). It suggests that in Thysanotus, the roots are a strong sink for carbohydrate. Given that the shoot tissue of T. tenellus, T. patersonii and T. juncifolius die back each year, during or before summer, development of underground storage would be critical for the regeneration of the plants the following season.

Regardless of the reason for the increased development of root tissue, T. tenellus had a significant growth of roots and increase in total dry matter to mycorrhizal infection under the conditions examined.

Discussion

Detailed examination of the fungal association of Thysanotus was required to determine whether the association was parasitic, commensal or symbiotic. Commensal associations are those where two different organisms exist together with no mutual influence. Parasitic associations have a unidirectional transfer of nutrients whereas in symbiotic associations there is considered to be mutual advantage (Abercrombie, Hickman & Johnson, 1973). However, there may be some degree of symbiosis in all forms of associated growth and there can be variation within different environments and at different stages of the association (Harley & Smith, 1983).

A mycorrhiza is a symbiotic association between soil-borne fungi and is characterised by two features. The morphology of the association is consistent from plant to plant within a species and there is an exchange of nutrients between symbionts. The fungi of mycorrhizas have a soil borne and a mycorrhizal phase each with a distinct

morphology. In VAM soil borne hyphae may swell to form an appressoria on the epidermis of the root or penetrate between the epidermal cells without significant change to the hyphae. The presence of arbuscules in cortical cells and vesicles in the root characterise VAM. In ectomycorrhizas there is often a characteristic flattening of hyphae on the root surface with the formation of typical aggregations of hyphae over the root surface and between cells of the epidermis. To determine if there is an exchange of nutrients within mycorrhizas, the common practice is to observe growth responses of the host to the presence of mycorrhizas, usually in soils with low levels of available nutrients.

The association between the roots of Thysanotus and soil borne fungi is a mycorrhiza as it accords with the features outlined above. The fungi of the association can also form either VAM or ectomycorrhizas with other hosts. The association has been found in the field on all plants examined so far. There was a growth response of Thysanotus to infection even though the roots rather than the shoots were most affected.

It would appear that mycorrhizas are essential for the survival of seedlings in soil of low fertility. Formation of mycorrhizas was associated with an increase of growth of seedlings, though there was a lack of spread of mycorrhizas unless a companion plant was present. Further work is required to elucidate the nutritional physiology of the association. However, from the data above, it is clear that the mycorrhizas of Thysanotus are not typical of either VAM or ectomycorrhizas.

Chapter 6

DISCUSSION

At Ferries McDonald Conservation Park there is a diverse range of plant species, some 40% of which are found in the more arid Central Australia. About 70% of the plants were found with VAM, approximately 30% with ectomycorrhizas, some 10% without mycorrhizas and the remainder found with other associations. Plants with ectomycorrhizas could also form VAM, annual species mostly being found with both, and perennial species usually with either type of association. The abundance of mycorrhizas suggest that they are important for the survival of plants in dry habitats.

Of the VAM fungi observed, there were no species that have not been observed in temperate climates. Even FE I has been isolated from soil collected from the Adelaide Hills. This suggests that, unlike plants, VAM fungi do not require special adaptations to survive aridity. Insufficient ectomycorrhizal fungi were identified to determine if any are typical of arid habitats.

The stratified distribution of VAM fungi, the slow germination of most spores and the absence of significant numbers of spores in soil at Ferries McDonald Conservation Park differs from the commonly studied agricultural systems. The species of VAM fungi in the upper soil profile had medium sized spores with slow germination so that hyphae in roots were important in the initiation of VAM after the wetting of soil. Hyphae in roots germinated readily at appropriate levels of soil water and became dormant when the soil dried out. As Gi. calospora, found at lower depths, germinated readily from all propagules but did not become dormant after one wet/dry cycle, there appear to be specific adaptations to local conditions by the VAM fungi. That is, those fungi found at the surface tolerate soil water stress associated with aridity, and a fungus found at depth avoids that soil water stress.

It is of interest that the plants in the park have reversed the adaptation. Annuals regenerate from seed at the soil surface avoiding water stress by only emerging when

conditions are suitable. Survival of annuals relies upon continuous growth till seed set. The perennials studied adapted to soil conditions with roots at depth, regrowing only while levels of soil water were adequate.

The presence of few spores of VAM fungi is possibly an adaptation to the presence of perennial growth, rather than aridity. Mosse and Bowen (1968) and Hayman and Stovold (1979) found few spores in soils collected under perennial vegetation. One possible explanation is that the slow germination of all spores reduces the competitiveness of a species.

It is not known if the hyphae in senescent roots are important in the long term survival of a VAM fungus. Given the delay in germination of spores, however, spores of species in the upper soil profile could function in this manner.

Annual and perennial plants were infected by specific VAM fungi according to the depth of the roots, and infection occurred from hyphae in senescent root pieces in most cases. Stratification of ectomycorrhizal fungi was not observed and infection by ectomycorrhizal fungi was associated with the nature of the propagule. FM Asco I regrew from small structures in the soil, possibly spores. FM Asco II and III, the Endogone sp. and Basidiomycetes regrew from old ectomycorrhizas and FM Asco II also regrew from sclerotia and rhizomorphs. The nature of the propagule was of importance to the source of initial infection of ectomycorrhizas. FM Asco I was of continued importance to annual species but was of only transitory importance to the perennial species examined. Presumably propagules of FM Asco I were widely distibuted in the soil, compared to the other ectomycorrhizal fungi, which were concentrated around roots of perennial species. This suggests that there is a specific selection of ectomycorrhizal fungi for association with annual and perennial species, with annuals associating with fungi that are widely distributed in soil and perennials associating with fungi that are distributed near the perennial root at the start of the growing season.

Two specific stresses associated with aridity at the park are high soil temperatures during summer and highly variable water content of soil. Both factors influenced the formation and/or spread of mycorrhizas. In annual plants, high soil

temperatures reduced the germination of seed, and the germination and infection of plants by ectomycorrhizal ascomycetes, but not VAM fungi. Spread of mycorrhizas also varied with temperature with VAM spreading readily from 16 to 30^{o}C, but FM Asco I being inhibited by high temperatures.

Soil water content was critical for the germination of seed and the reemergence of roots but not so critical for the germination and spread of mycorrhizal fungi, such that conditions suitable for germination of fungi can occur in the absence of root growth. While the 'cost' of germination and growth of fungal propagules has not been examined, it would appear that fungi might not survive many wet/dry cycles due to limited reserves. That there appear to be so few propagules in reserve, suggests that the survival of mycorrhizal fungi is not significantly affected by a semiarid environment.

The consequence of variable soil water and temperature is a highly complex interaction between various mycorrhizal fungi and their hosts. There appear to be few factors that distinguish between mycorrhizas in a dry and a temperate climate. The species of mycorrhizal fungi found at the park are also found elsewhere, the types of mycorrhizal associations found at the park are also found in temperate climates, though there were more ectomycorrhizas found at the park than have been reported to occur to occur in arid California (Bethlenfalvay et al, 1984). Even the unusual mycorrhizal associations observed on Thysanotus juncifolius, T. patersonii and Wurmbea dioica are observed on these species in wetter climates.The lack of difference between the park and more temperate habitats may reflect the close proximity of the park to the Adelaide Hills, but it is hard to see why if climate influences mycorrhizal associations.

Thus it is argued that the stresses of aridity that are associated with numerous adaptations in plants, do not have a significant affect on mycorrhizal fungi at Ferries McDonald Conservation Park and that mycorrhizas have the same importance to plants in dry as in temperate climates.

Appendix 1

Plant Species of Ferries-McDonald Conservation Park (combined from the list of Cleland (1955) and own observations marked *). Authorities of a species have been given only where the identity has been determined recently. Families of the Monocots according to Dahlgren, Clifford and Yeo (1985) and the rest according to Jessop and Toelken (1986).

Cuppressaceae
- Callitris canescens
- C. columellaris F. Muell.*
- C. preissii

Anthericaceae
- Thysanotus iucifolius (Salisb.) J.H. Willis & Court*
- T. patersonii R. Br.*
- Tricoryne elatior R. Br.*

Asphodelaceae
- Bulbine semibarbarta (R. Br.) Haw.*
- Laxmannia sessiliflora Decne.*

Colchicaceae
- Wurmbea dioica (R. Br.) F. Muell.*

Cyperaceae
- Gahnia deusta
- G. lanigera
- Lepidesperma carphoides
- L. viscidum
- Schoenus racemosus

Dianellaceae
- Dianella revoluta R. Br.*

Hypoxidaceae
- Hypoxis pusilla

Iridaceae
- Gynandriris setifolia (L. f.) Foster*
- Romulea minutiflora

Orchidaceae
- Acianthus exsertus
- A. reniformis
- Caladenia cardiochila
- C. deformis
- C. dilatata
- C. filamentosa
- Diuris palustris
- Microtis unifolia
- Pterostylis cycnocephala
- P. mutica
- P. nana
- P. plumosa
- P. pusilla
- P. robusta
- P. vittata
- Thelymitra antennifera
- T. aristata

Poaceae
- Aira caryophyllea L. *
- Avellinia michelii (Savi) Parl.*
- Cynodon dactylon
- Danthonia caespitosa Gaudich.*
- Lophochloa pumila
- Neurachne alopecuroides

Pentaschistis airoides
Stipa elegantissima Labill.*
S. semibarbata
Vulpia myuros (L.) C. Gmelin*

Restionaceae
Hypolaena fastigiata

Xanthorrhaceae
Lomandra dura
L. effusa
L. juncea (F. Muell.) Ewart*
L. leucocephala
L. micranthra

Aizoaceae
Carprobrotus modestus S.T. Blake*

Amaranthaceae
Ptilotus spathulatus (R. Br.)Poiret*

Apiaceae
Daucus glochidiatus (Labill.) Fischer, C. Meyer & Ave Lall.*
Hydrocotyle pilifera Turcz.*
Trachymene(?) pusillus*

Asteraceae
Actinobole uliginosum (A. Gray) H. Eichler*
Arctotheca calendula (L.) Levyns*
Blenospora drummondii A. Gray*
Brachycome ciliarus (Labill.) Less.*
B. lineariloba (DC.) Druce*
B. perpusilla (Steetz) J. Black*
Carduus tenuiflorus
Carthamus lanatus
Cassinia laevis R. Br.*
Centaurea melitensis
Dittrichia graveolens
Gnaphalium involucratu Forster*
Haeckeria punctulata
Hedypnois cretica
Helichrysum apiculatum (Labill.) D. Don*
H. bilobum
H. catadromum
H. leucopsidium DC.*
H. obtusifolium F. Muell. & Sonder ex Sonder*
Helipterum demissum (A. Gray) Druce*
H. pygmaeum (DC.) Benth.*
Hypochoeris glabra
H. radicata
Microseris scapigera
Millotia tenuifolia Cass.*
Olearia ciliata (Benth.) F. Muell. ex Benth.*
O. lepidophylla
O. picridifolia
O. ramulosa (Labill.) Benth.*
Podolepis rugata Labill.*
P. tepperi (F. Muell.) D. Cooke*
Podotheca angustifolia (Labill.) Less.*
Polygonolepis muelleriana
Pseudognaphalium luteo-album (L.) Hilliard & B.L. Burtt*
Rutidosis multiflora
Senecio glossanthus (Sonder) Belcher*
S. lautus Forster f. ex. Willd.*
S. quadridentatus

Sonchus oleraceus L.*
Toxanthus muelleri (Sonder) Benth.*
Vittadinia dissecta (Benth.) N. Burb.*

Brassicaceae
Brassica tournefortii Gouan*
Cardamine sp.*
Lepidium hyssopifolium
Stenopetalum lineare R. Br. ex DC.*
S. sphaerocarpum

Campanulaceae
Lobelia gibbosa Labill.*
Wahlenbergia communis Carolin*
W. gracilenta Loth.*

Caryophyllaceae
Arenaria serpylifolia
Petrorhagia velutina (Guss.) P. Ball*
Silene nocturna L.*

Casuarinaceae
Allocasuarina muelleriana (Miq.) L. Johnson*
A. pusilla

Chenopodiaceae
Rhagodia candolleana Moq.*

Crassulaceae
Crassula colurata (Nees) Ostenf.*

Dillenaceae
Hibbertia stricta (R. Br. ex DC.) F. Muell.*
H. virgata R. Br. ex DC.*

Droseraceae
Drosera macrantha Endl.*
D. whittakeri Planchon*

Epacridaceae
Acrotriche cordata
A. depressa
Astroloma conostephioides
A. humifusum (Cav.) R. Br.*
Brachyloma ericoides
Leucopogon clelandii Cheel*
L. rufus Lindley*
Styphelia exarrhena

Ehretiaceae
Halgania cyanea

Euphorbiaceae
Adriana klotzchii
Bertya mitchellii
Beyeria leschenaultii (DC.) Baillon*
Poranthera microphylla Brongn.*

Fabaceae
Daviesia genistifolia
Dillwinia hispida Lindley*
D. uncinata
Kennedya prostrata
Medicago polymorpha L.*
Phyllota remota
Trifolium arvense
T. campestra

Gentianaceae
Centaurium erythrea Rafn.*
Sebea ovata

Geraniaceae
- Erodium botrys
- E. cicutarium

Goodeniaceae
- Dampiera rosemarinifolia Schldl.*
- Goodenia affinis (Vriese) Vriese*
- G. robusta (Benth.) Krause*

Haloragaceae
- Glischrocaryou behrii (Schldl.) Orch.*

Lamiaceae
- Marrubium vulgare
- Prostanthera aspalathoides Cunn. ex Benth.*
- Salvia verbenaca
- Westringia dampieri R. Br.*
- W. rigida

Lauraceae
- Cassytha glabella
- C. melantha
- C. pubescens

Linaceae
- Linum marginale

Loganaceae
- Logania linifolia
- Mitrasacme paradoxa

Mimosaceae
- Acacia brachybotrya
- A. calamifolia
- A. microcarpa
- A. rhetinocarpa
- A. rigens Cunn. ex Don*
- A. rupicola
- A. spinescens Benth.*

Myoporaceae
- Eremophila crassifolia
- E. glabra
- Myoporum platycarpum

Myrtaceae
- Baeckea behrii (Schldl.) F. Muell.*
- B. crassifolia Lindley*
- B. ericaea
- Callistemon macropunctatus
- Calytrix tetragona Labill.*
- Eucalyptus fasciculosa F. Muell.*
- E. incrassata Labill.*
- E. oleosa F. Muell. ex Miq.*
- E. rugosa R. Br. ex Blakely*
- Kunzea pomifera
- Leptospermum coriaceum
- L. myrsinoides Schldl.*
- Melaleuca acuminata F. Muell.*
- M. lanceolata Otto*
- M. uncinata R. Br.*

Pittosporaceae
- Billardiera cymosa F. Muell.*
- Bursaria spinosa Cav.*
- Cheiranthera linearis

Plantaginaceae
- Plantago drummondii Decne.*

Polygalaceae
- Comesperma calymega Labill.*
- C. volubile

Polygonaceae
- Muehlenbeckia adpressa

Portulucaceae
- Calandrinia corrigioloides
- C. granulifera Benth.*
- C. volubilis Benth.*

Primulaceae
- Anagallis arvensis L.*
- A. femina

Proteaceae
- Adenanthos terminalis
- Banksia ornata
- Grevillea ilicifolia (R. Br.) R. Br.*
- G. lavandulaceae Schldl.*
- Hakea muelleriana J. Black*

Ranunculaceae
- Clematis microphylla DC.*

Rhamnaceae
- Cryptandra amara
- C. leucophracta
- C. tomentosa Lindley*
- Pomaderris obcordata Fenzl.*
- P. oraria
- Spyridium eriocephalum Fenzl.*
- S. subochreatum

Rubiaceae
- Galium murale
- Opercularia varia

Rutaceae
- Boronia coerulescens F. Muell.*
- B. inornata
- Correa schlechtendalii Behr*
- Eriostemon pungens Lindley*
- Microcybe pauciflora
- Phebalium bullatum J. Black*

Santalaceae
- Choretrum glomeratum
- Exocarpus sparteus R. Br.*
- Santalum acuminatum (R. Br.) A. DC.*
- S. murrayanum (T.L. Mitchell) C. Gardner*

Sapinadaceae
- Dodoneaea bursariifolia
- D. hexandra
- D. humilis
- D. stenozyga

Scrophulariaceae
- Veronica hillebrandii
- Zaluzianskya divaricata (Thunb.) Walp.*

Solonaceae
- Nicotiana glauca

Stackhousiaceae
- Stackhousia monogyna

Sterculiaceae
- Lasiopetalum baueri
- L. behrii F. Muell.*
- Thomasia petalocalyx

Thymelaeaceae
- Pimelea glauca R. Br.*
- P. octophylla
- P. serpyllifollia

Urticacea
- Urtica urens

Violaceae
- Hybanthus floribundus (Lindley) F. Muell.*

Zygophyllaceae
- Nitraria billardirei
- Zygophyllum glaucescum

Appendix II. Rainfall data of the farm "Rocklea", situated on the southern boundary of Ferries McDonald Conservation Park for the years 1983, 1984, 1985 and January to May of 1986 (mm).

Day	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
1983													
1			2	6				6					
2					13		5						
3			10						6				
4			11										
5						4			8				
6												9	
7							6		1			3	
8									5				
9				15									
10				18									
11									2				
12	2			7		4	4			3			
13									8				
14					6					4	7		
15			8		4				2	35			
16								15					
17													
18								12					
19										8			
20							4			3			
21										1			
22			18										
23	2		52										
24													
25				11		1		17					
26	2			2							2		
27							11	7					
28							6			1			
29					5		4						
30					4		10	6					
31												8	
Tot	6	0	101	59	32	9	50	63	32	55	9	20	436

Appendix II, cont.

Day	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
1984													
1	23										10		
2													
3										16			
4							10		13				
5								10					
6													
7	1			13									
8													
9													
10			3				9	6			14		
11								13					
12													
13			5				3						
14			2								2	1	
15				2		3							
16					4	6	12	3					
17								5	9	2			
18				4				11					
19					3								
20				3								1	
21								5					
22					5				4				
23					1								
24							12						
25			7				1	10					
26	6												
27													
28								3					
29		2											
30							4						
31													
Tot	30	2	17	22	13	9	51	66	26	18	26	2	282

Appendix II, cont.

Day	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
1985													
1													
2						3							
3													
4													
5						18							
6								8					
7												10	
8					3			16				10	
9								4	3		7	3	
10								1				3	
11						1				8			
12								1					
13									22				
14				27	4			3					
15			1	6					1				
16			67				10	8		3			
17					5		1						
18				4									
19													
20						13							
21													
22							4	14		7			
23										13	1		
24								2					
25											3		
26								3	3				
27									5			1	
28					2		1	1					
29													
30					3		1						
31												1	
Tot	0	0	68	37	17	35	17	61	46	31	11	28	351

Appendix II, cont.

Day	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1												
2												
3												
4												
5												
6												
7												
8												
9					5							
10												
11												
12												
13	1											
14												
15				13								
16				6								
17				3								
18												
19												
20												
21												
22												
23												
24				3								
25				1								
26												
27												
28												
29												
30												
31												
Tot	1	0	0	26	5							

Appendix 3. Maximum and minimum daily temperatures recorded using the soil thermograph, from 19.1.84 to 28.2.84 at 2 and 12cm down the soil profile under a bare surface and from 29.11.84 to 13.2.85 at 2cm under a litter layer or under a bare surface (°C).

Date of recording	2cm Maximum	2cm Minimum*	12cm Maximum	12cm Minimum
19.1.84	56	21	34	23
20	56	21	34	24
21	44	21	31	21
22	57	21	34	23
23	62	21	37	26
24	49	21	33	24
25	21	21	23	20
26	27	21	27	17
27	32	21	29	17
28	45	20	37	22
29	41	21	34	22
30	43	21	32	22
31	44	21	31	19
1.2.84	53	21	35	21
2	58	21	37	23
3	61	21	38	26
4	56	21	36	24
5	60	21	38	23
6	37	21	32	25
7	52	21	35	23
8	51	21	34	23
9	48	21	32	22
10	59	21	36	23

Appendix 3, cont.

Date of recording	2cm Maximum	2cm Minimum	12cm Maximum	12cm Minimum
11	52	21	34	24
12	49	21	34	24
13	42	21	31	22
14	50	21	33	22
15	50	21	33	24
16	51	21	33	23
17	51	21	34	23
18	56	21	36	25
19	58	21	38	23
20	52	21	37	26
21	45	21	32	23
22	53	21	35	26
23	36	21	30	23
24	47	21	32	22
25	50	21	33	22
26	56	21	35	26
27	21	21	26	18
28	27	21	27	24

* - recorder malfunctioning

Date of recording	Bare surface Maximum	Bare surface Minimum	Litter cover Maximum	Litter cover Minimum
29.11.84	44	11	26	14
30	48	9	28	13
1.12.84	57	11	31	16

Appendix 3, cont.

Date of recording	Bare surface		Litter cover	
	Maximum	Minimum	Maximum	Minimum
2.12.84	42	9	26	14
3	32	11	22	15
4	48	15	23	18
5	55	14	27	12
6	52	13	28	17
7	60	14	31	13
8	61	19	33	21
9	63	18	35	22
10	36	10	27	17
11	48	11	24	16
12	52	16	27	18
13	42	15	26	18
14	56	12	28	17
15	51	11	28	16
16	47	11	26	16
17	61	15	32	14
18	55	13	29	18
19	35	13	24	17
20	40	11	24	16
21	54	17	28	19
22	60	13	32	18
23	60	17	33	21
24	42	18	30	21
25	43	12	26	17
26	53	10	28	16
27	57	12	21	18

Appendix 3, cont.

Date of recording	Bare surface		Litter cover	
	Maximum	Minimum	Maximum	Minimum
28.12.84	63	15	24	20
29	65	19	36	22
30	37	17	26	20
31	37	15	25	19
1.1.85	52	14	28	19
2	30	10	22	15
3	35	13	23	15
4	36	16	22	17
5	35	14	22	15
6	48	12	25	15
7	57	14	28	17
8	58	15	29	19
9	50	18	27	19
10	53	17	28	19
11	57	13	31	18
12	62	16	34	20
13	70	21	38	24
14	61	18	36	23
15	53	15	30	20
16	48	12	28	18
17	47	18	26	16
18	53	20	29	17
19	32	21	24	17
20	59	17	32	21
21	61	18	35	22
22	48	17	29	21

Appendix 3, cont.

Date of recording	Bare surface		Litter cover	
	Maximum	Minimum	Maximum	Minimum
23.1.85	52	12	30	18
24	55	11	31	18
25	61	14	31	20
26	66	14	32	21
27	54	13	?	?
28	?	?	?	?
29	?	?	?	?
30	?	?	?	?
31	48	8	29	17
1.2.85	54	11	32	18
2	60	14	33	?
3	56	16	?	?
4	?	16	?	?
5	?	?	?	?
6	?	?	?	?
7	43	8	26	15
8	50	9	29	16
9	52	11	32	18
10	53	15	32	19
11	29	15	?	?
12	52	13	?	?
13	46	14	?	?

? - ink dried on nib of recorder, no mark left on paper.

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